PROCESS FOR PREPARING 6-ALKYLIDENE PENEM DERIVATIVES

This application claims priority from copending application Serial No. 10/427,666, filed May 1, 2003, which claims the benefit of provisional application 60/377,048 filed May 1, 2002, the entire disclosure of each hereby being incorporated by reference.

FIELD OF THE INVENTION

This invention relates to a novel process for the production of 6-alkylidene penem derivatives that can be important as broad spectrum β-lactamase inhibitors and anti-bacterial agents.

BACKGROUND OF THE INVENTION

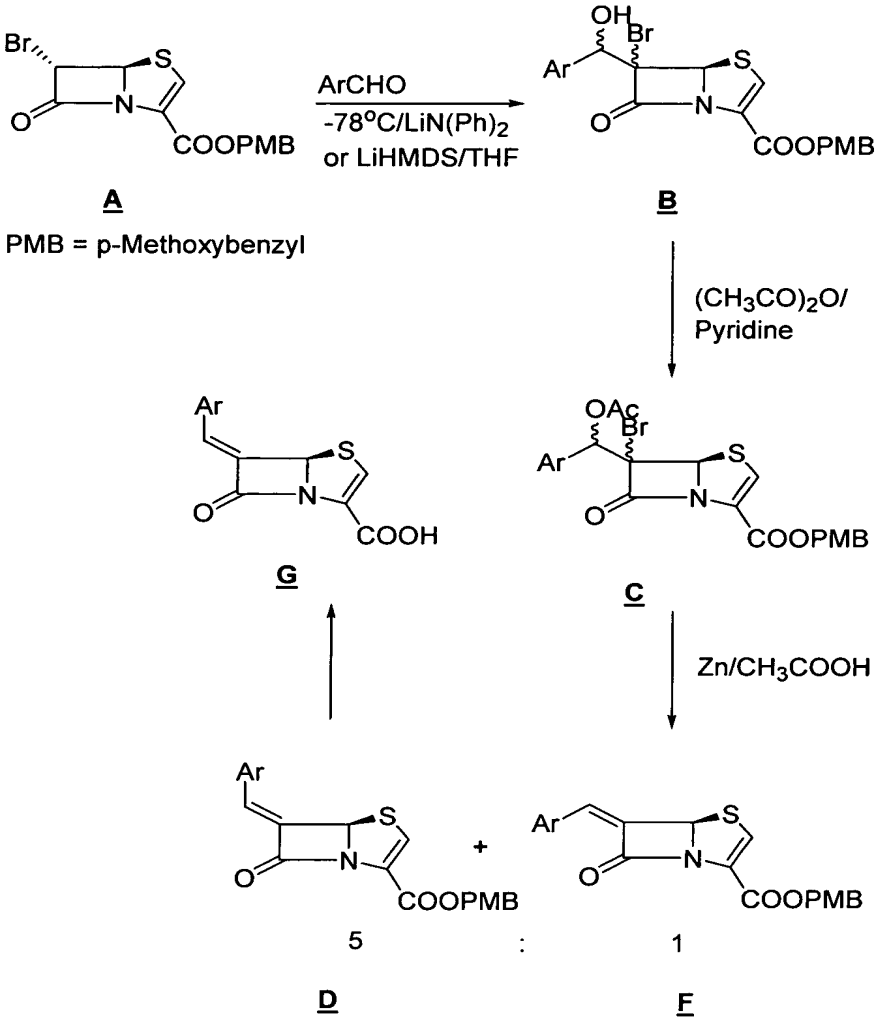
β-Lactamases are enzymes produced by the bacteria, that hydrolyze β-lactam antibiotics and as such serve as the primary cause of bacterial resistance. Penicillins and cephalosporins are the most frequently and widely used β-lactam antibiotics in the clinic. However, the development of resistance to β-lactam antibiotics by different pathogens has had a damaging effect on maintaining the effective treatment of bacterial infections. (Coleman, K. *Expert Opin. Invest. Drugs* **1995,** 4, 693; Sutherland, R. *Infection* **1995,** 23 (4) 191; Bush, K, *Cur. Pharm. Design* **1999,** 5, 839-845) The most significant known mechanism related to the development of bacterial resistance to the β-lactam antibiotics is the production of class-A, class-B and class-C serine β-lactamases. These enzymes degrade the β-lactam antibiotics, resulting in the loss of antibacterial activity. Class-A enzymes preferentially hydrolyze penicillins where as Class-C lactamases have a substrate profile favoring cephalosporin hydrolysis. (Bush, K.; Jacoby, G.A.; Medeiros, A.A. *Antimicrob. Agents Chemother.* **1995,** 39, 1211). To date over 250 different β-lactamases have been reported (Payne, D.J,: Du, W and Bateson, J.H. *Exp. Opin. Invest. Drugs* **2000,** 247.) and there is a need for a new generation of broad spectrum β-lactamases inhibitors. Bacterial resistance to these antibiotics could be

greatly reduced by administering the β-lactam antibiotics in combination with a compound which inhibits these enzymes.

The commercially available β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam are all effective against class-A producing pathogens. The mechanism of inactivation of class-A β-lactamases (such as PCI and TEM-1) has been elucidated. (Bush, K.; *Antimicrob. Agents Chemother.* **1993**, 37, 851; Yang, Y.; Janota, K.; Tabei, K.; Huang, N.; Seigal, M.M.; Lin, Y.I.; Rasmussen, B.A. and Shalaes, D.M. *J. Biol. Chem.* **2000**, 35, 26674-26682) However, these compounds are ineffective against class-C producing organisms. Clavulanic acid is used in combination with amoxicillin and ticarcillin; similarly sulbactam with ampicillin and tazobactam with piperacillin.

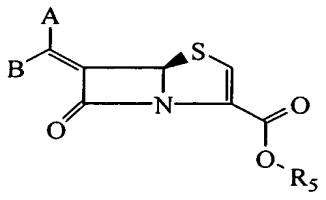
EP 0 041 768A, EP 0 120 613 A, EP 0 150 781, EP 0210065, WO 87/00525, EP 0 003 960 B1, GB 2 042 514 A, GB 2 042 515 A, EP 0 087 792 A, EP 0115 308, A, GB 2 124614B, EP 0 150984 A, EP 0087792 , EP 0115308, EP 81 301683.9, EP 84 301255.0, EP 85100520.7, EP 85100521.5, EP 86305585.1, EP 86 305584, EP 88 311786.3, EP 88 311787.1, EP 87 300193.7, WO 93/03042, WO 94/10178, WO 95/28935 and WO 95/17184 disclose 6-(substituted methylene)-penem as β-lactamase inhibitors. EP 0 232 966 B1 discloses a process for preparing 6-(substituted methylene)-penems and their intermediates. According to this patent, the compounds of general formula **I** were prepared by a four step process starting from p-methoxybenzyl(5R,6S) 6-bromopenem-3-carboxylate **A** and an aldehyde in the presence of pyrophoric reagents such as n.butyllithium/diphenyl amine or lithium bis(trimethylsilyl)amide at –78°C (Scheme 1). The third step of this process, namely the reductive elimination step, gave Z and E compounds, isomers **D** and **F** in the ratio of 5:1 respectively.

Scheme 1



SUMMARY OF THE INVENTION

The present invention relates to a process for the preparation of compound of formula **I**



I

wherein

one of A and B denotes hydrogen and the other is aryl optionally substituted with one or two R_2, heteroaryl optionally substituted with one or two R_2, a fused bicyclic heteroaryl optionally substituted with one or two R_2, fused tricyclic heteroaryl optionally substituted with one or two R_2, cycloalkyl optionally substituted with one or two R_2, alkyl optionally substituted with one or two R_2, alkenyl optionally substituted with one or two R_2, alkynyl optionally substituted with one or two R_2, saturated or partially saturated heteroaryl optionally substituted with one or two R_2;

R_5 is H, an in vivo hydrolyzable ester selected from the group C1 –C6 alkyl, C5 – C6 cycloalkyl, CHR_3OCOC1-C6 or a salt selected from the group consisting of Na, K, and Ca;

R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl having 1 to 2 double bonds, optionally substituted C2-C6 alkynyl having 1 to 2 triple bonds, halogen, cyano, $N\text{-}R_6R_7$, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$- optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted alkylaryloxyalkylamines;

R_3 is hydrogen, C1-C6 alkyl, C3 – C6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl;

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkyl aryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkyl heteroaryl, R_6 and R_7 can be taken together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms such as N-R_1, O, S=$(O)_n$ n = 0-2;

which process comprises the following steps:

Step 1: Dissolving 6-aminopenicillanic acid **20** in an organic solvent (preferably methanol or THF) and water to form the 6-bromo derivative in **21** the presence of 48% w/w hydrobromic acid at –10° C to –30°C and sodium or potassium nitrite solution. The 6-bromopenicillanic acid **21** derivative either can be isolated or *insitu* converted to the p-Nitrobenzyl 6-brompenicillanate **22** using 4-nitrobenzylbromide in the presence of organic bases or inorganic bases (preferably sodium or potassium carbonate) in an organic solvent (preferably THF or DMF).

Step 2: The product 4-nitrobenzyl 6-bromopenicillanate **22** obtained by the process outlined in step 1 can be isolated or be transformed to 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** in the same pot (i.e Step 1; sequential formation) by oxidizing **22** to 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** using any known oxidizing agents such as 3-chloroperoxybenzoic acid (mcpba) or hydrogen peroxide.

Step 3: The product from step 2, namely 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** can be converted to 4-nitrobenzyl(2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-3-enoate **24** by refluxing 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** with 2-mercaptobenzothiazole in an aromatic solvent (preferably toluene or xylene).

Step 4: The product from step 3, namely 4-nitrobenzyl(2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-3-enoate **24** can be dissolved in an organic solvent (preferably Toluene or Xylene) and upon reaction with an organic tertiary base (preferably triethylamine) at ambient temperature gave 4-nitrobenzyl-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-2-enoate **25**.

Step 5: The product from step 4, namely 4-nitrobenzyl-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-2-enoate **25** can be converted to **26** 4-nitrobenzyl 2-[(3S,4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate by carrying out the reaction in an aromatic organic solvent (preferably toluene) in the presence of an organic acid (preferably formic acid) , acetic anhydride/ organic tertiary base (preferably pyridine) and trialkyl or triaryl phosphine (preferably triphenylphosphine) at –10° C to –30° C (preferably about –15 to 20° C).

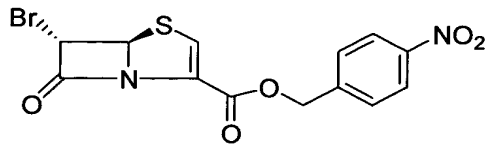
An alternative embodiment of the present invention relates to the sequential conversion of compound **23** to **26** without isolating the intermediates:

Product from Step 2 namely 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** may be reacted with mercaptobenzothiazole in refluxing aromatic organic solvent (preferably Toluene) preferably for 1 to 3 hrs and treated with triethylamine at 0 to –20° C for 3 to 4 hrs. After this treatment, reaction mixture is charged with an organic acid (preferably formic acid) and an anhydride (acetic anhydride), an organic tertiary base (preferably pyridine) and a trialkyl or triaryl phosphate sequentially at –10° C to -40° C.

Step 6: The product 4-nitrobenzyl 2-[(3S,4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate **26** from either step 5 or from the sequential conversion was taken up in an organic solvent (preferentially ethyl acetate) at –70°C to –90° C and ozonized oxygen was passed through it for 3 to 4 hrs followed by intramolecular cyclization using a phosphite reagent (preferably trimethyl phosphite). The product

4-nitrobenzyl (5R,6S)-6-bromopenem-3-carboxylate **16** was crystallized from ethylacetate:hexane.

The present invention also relates to the compound represented by the following formula (**16**) :



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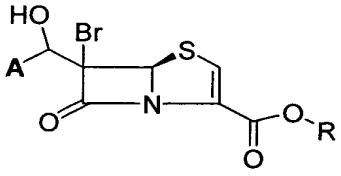
An above mentioned process (Step 1 to Step 6) for the preparation of the compound represented by the formula **16** is an intermediate useful for the preparation of 6-(substituted methylene)penems of general formula **I**.

The compound represented by the formula **16** is a crystalline derivative (X-ray powder diffraction parameters are given in the Table 2)

The crystalline nature of this intermediate imparts stability and thereby increases the shelf-life time. The stability data of the compound of the formula **16** is given in Table 1.

Step 7: Reaction of 4-nitrobenzyl (5R,6S)-6-bromopenem-3-carboxylate **16** with the appropriately substituted aldehydes (defind as before) to effect the aldol condensation step can be carried out in the presence of a Lewis acid (preferably anhydrous $MgBr_2$ or $MgBr_2$:



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etherate) and a organic tertiary base (preferably Et_3N, pyridine, dimethylamino pyridine (DMAP), or diisopropylethylamine trialkylamine) in an aprotic polar organic solvent(s) (preferably THF and/or acetonitrile) at temperature –10° C to –40°C. The

intermediate aldol products of general formula **18** are functionalized with an acid chloride or anhydride to an acetate, triflic anhydride to a triflate or tosylchloride to a tosylate at 0°C to – 10° C in the same pot; or, if formulae **18** is isolated, it can be converted to a halogen derivative by reacting **18** with tetrahalomethane and triphenyl phosphine at room temperature in a suitable organic solvent preferably CH_2Cl_2.

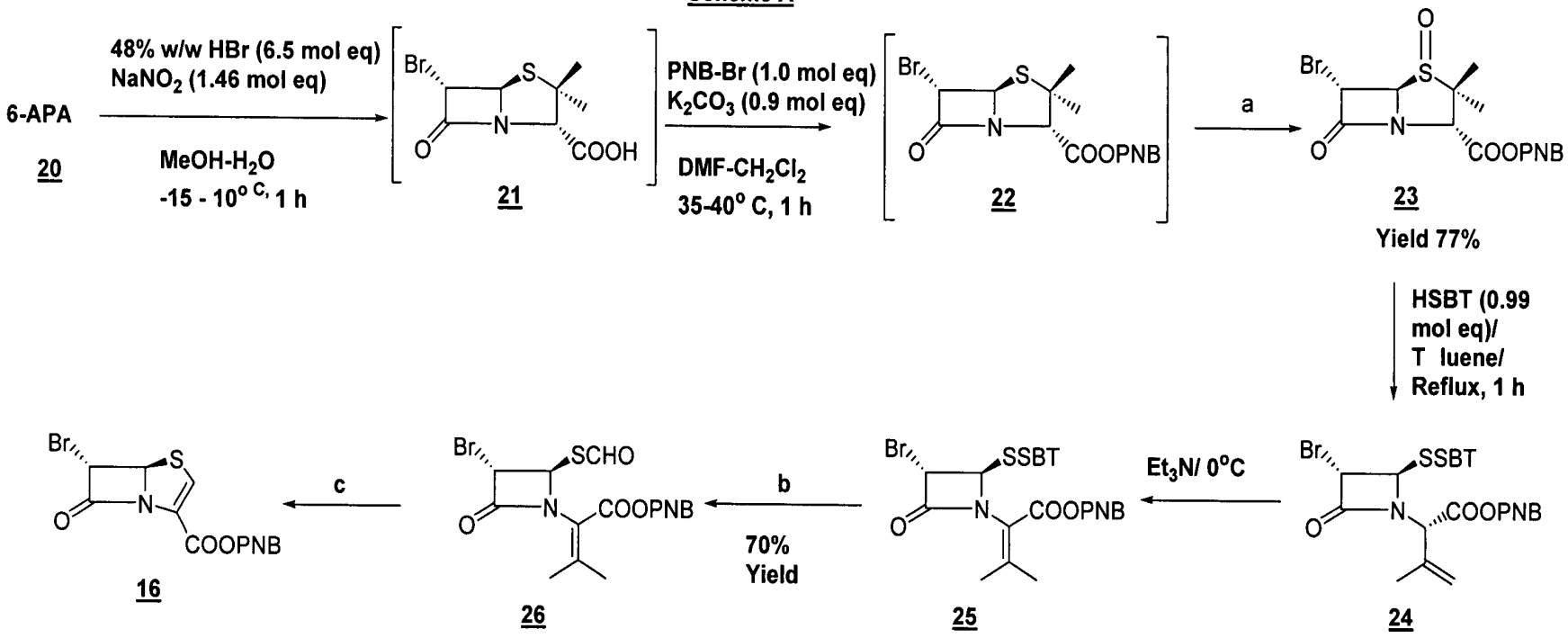
Step 8: The final step, namely the reductive elimination step to yield the compound of the general formula **I** can be carried out by dissolving the aldol product mixture in an organic solvent (preferably THF/ acetonitrile) and at a pH range of 6.0 – 8.5, preferably 6.5 to 7, pH phosphate buffer and activated metal such as zinc, tin or aluminum at ambient temperature (preferably at 20° C to 35°C). The product as a alkalie metal salt can be purified by a reverse phase resin column chromatography.

Alternatively Step 7 and Step 8 can be carried out sequentially in the same pot without isolating the aldol intermediate.

The final step, namely the reductive elimination step can be carried out by dissolving the aldol product in an organic solvent (such as THF or acetonitrile) and 6.5-7.0 phosphate buffer and hydrogenating over Pd/C at 10 to 100 psi (preferably at 40 psi) pressure.

A representative example is shown in Scheme A which illustrates the present invention. In Scheme A suitable reaction conditions are shown, however, other reaction conditions may be used without departing from the scope of the invention. For example shorter or longer reaction times may be employed; generally the longer the reaction time the more complete the reaction.

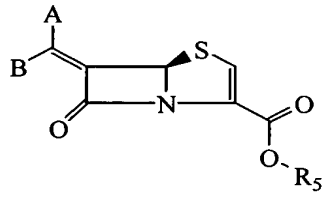
Scheme A



PNB= *para*-Nitrobenzyl. a: mcpba (0,89 eq)/ DMF-CH_2Cl_2/ 0° C, 20 min. Crystallization; b: (1)HCOOH (4.4 mol eq)/ AC_2O (4.4 mol eq)/ Pyridine (1.03 mol eq)/ MeCN, -15°C, 5 min; (2) Ph_3P (1.02 mol eq) -15°C, 1 h. (3) Column; (4) Crystallization; c: (1) O_3, AcOEt, -70° C, 1.75 hrs; (2) $P(OMe)_3$ (4.8 mol eq), Reflux, 45 min; (3) Crystallization

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a novel process for the preparation of a compound of the formula I.



I

wherein:

One of A and B denotes hydrogen and the other is an aryl optionally substituted with one or two R_2, heteroaryl optionally substituted with one or two R_2, a fused bicyclic heteroaryl optionally substituted with one or two R_2, fused tricyclic heteroaryl optionally substituted with one or two R_2, cycloalkyl optionally substituted with one or two R_2, alkyl optionally substituted with one or two R_2, alkenyl optionally substituted with one or two R_2, alkynyl optionally substituted with one or two R_2, saturated or partially saturated heteroaryl optionally substituted with one or two R_2.

R_5 is H, an in vivo hydrolyzable ester such as C1 –C6 alkyl, C5 – C6 cycloalkyl, CHR_3OCOC1-C6 or salts such as Na, K, or Ca.

R_1 is H, optionally substituted -C1-C6 alkyl, optionally substituted -aryl, optionally substituted -heteroaryl or mono or bicyclic saturated heterocycles, optionally substituted –C3-C7 cycloalkyl, optionally substituted -C3-C6 alkenyl, optionally substituted -C3-C6 alkynyl with the proviso that both the double bond and the triple bond should not be present at the carbon atom which is directly linked to N; optionally substituted -C1-C6 per fluoro alkyl, $-S(O)_p$ optionally substituted alkyl or aryl where p is 2, optionally substituted -C=Oheteroaryl, optionally substituted -C=Oaryl, optionally substituted -C=O (C1-C6) alkyl, optionally substituted -C=O (C3-C6) cycloalkyl, optionally substituted -C=O mono or bicyclic saturated heterocycles, optionally substituted C1-C6 alkyl aryl, optionally substituted C1-C6 alkyl heteroaryl,

optionally substituted aryl-C1-C6 alkyl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, -$CONR_6R_7$, -$SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted -alkyl-O-alkyl-aryl, optionally substituted -alkyl-O-alkyl-heteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkyl aryloxyheteroaryl, optionally substituted alkyl aryloxy alkylamines, optionally substituted alkoxy carbonyl, optionally substituted aryloxy carbonyl, optionally substituted heteroaryloxy carbonyl. Preferred R_1 groups are H, optionally substituted alkyl, optionally substituted aryl, -C=O(C1-C6)alkyl, C3-C6alkenyl, C3-C6alkynyl, optionally substituted cycloalkyl, SO_2alkyl, SO_2aryl, optionally substituted heterocycles, -$CONR_6R_7$, and optionally substituted heteroaryl.

R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl having 1 to 2 double bonds, optionally substituted C2-C6 alkynyl having 1 to 2 triple bonds, halogen, cyano, N-R_6R_7, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$- optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6 alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally substituted aryloxyalkyl,

optionally substituted heteroaryloxyalkyl, optionally substituted alkylaryloxyalkylamines. Preferred R_2 groups are H, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted heteroaryl, halogen, CN, hydroxy, optionally substituted heterocycle, $-CONR_6R_7$, $COOR_6$, optionally substituted aryl, $S(O)_q$-alkyl, and $S(O)_q$-aryl.

R_3 is hydrogen, C1-C6 alkyl, C5 – C6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl.

R_4 is H, optionally substituted C1-C6 alkyl, one of R_4 is OH, C1-C6 alkoxy, -S-C1-C6 alkyl, $COOR_6$, $-NR_6R_7$, $-CONR_6R_7$; or R_4R_4 may together be =O or R_4R_4 together with the carbon to which they are attached may form a spiro system of five to eight members with or without the presence of heteroatoms selected N, O, S=(O)n (where n =0 to 2), N-R_1;

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkyl aryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkyl heteroaryl, R_6 and R_7 can be together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms such as N-R_1, O, $S=(O)_n$ n = 0-2.

Chemical Definitions

The term alkyl means both straight and branched chain alkyl moieties of 1-12 carbons, preferably of 1-6 carbon atoms.

The term alkenyl means both straight and branched alkenyl moieties of 2-8 carbon atoms containing at least one double bond, and no triple bond, preferably the alkenyl moiety has 1 or two double bonds. Such alkenyl moieties may exist in the E or Z conformations; the compounds of this invention include both conformations. In the case of alkenyl, hetero atoms such as O, S or N-R_1 should not be present on the carbon that is bonded to a double bond;

The term alkynyl includes both straight chain and branched alkynyl moieties containing 2-6 carbon atoms containing at least one triple bond, preferably the alkynyl moiety has one or two triple bonds. In the case of alkynyl, hetero atoms such as O, S or N-R_1 should not be present on the carbon that is bonded to a double or triple bond;

The term cycloalkyl refers to a alicyclic hydrocarbon group having 3-7 carbon atoms. The term perfluoroalkyl is used herein to refer to both straight- and branched-chain saturated aliphatic hydrocarbon groups having at least one carbon atom and two or more fluorine atoms. Examples include CF_3, CH_2CF_3, CF_2CF_3 and $CH(CF_3)_2$. The term halogen is defined as Cl, Br, F, and I.

If alkyl, alkenyl, alkynyl, or cycloalkyl is "optionally substituted", one or two of the following are possible substituents: nitro, -aryl, -heteroaryl, alkoxycarbonyl-, -alkoxy, -alkoxy-alkyl, alkyl-O-C2-C4alkyl-O-, -cyano, -halogen, -hydroxy, -N-R_6R_7, -trifluoromethyl, -trifluoromethoxy, arylalkyl, alkylaryl, R_6R_7N-alkyl-, HO-C1-C6-alkyl-, alkoxyalkyl-, alkyl-S-, -SO_2N-R_6R_7, -SO_2NHR_6, -CO_2H, $CONR_6R_7$, aryl-O-, heteroaryl-O-, -$S(O)_s$-aryl (where s = 0 –2), -alkyl-O-alkyl-NR_6R_7, -alkyl-aryl-O-alkylN-R_6R_7, C1-C6alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy-alkyl-O-, R_6R_7N-alkyl-, and -$S(O)_s$-heteroaryl (where s = 0 –2); Preferred substitutents for alkyl, alkenyl, alkynyl, and cycloalkyl include: halogen, nitro, aryl, heteroaryl, alkoxycarbonyl-, alkoxy, -alkoxy-alkyl, -cyano, hydroxy, and -N-R_6R_7.

Aryl is defined as an aromatic hydrocarbon moiety selected from the group: phenyl, α-naphthyl, β-naphthyl, biphenyl, anthryl, tetrahydronaphthyl, fluorenyl, indanyl, biphenylenyl, acenaphthenyl, groups.

Heteroaryl is defined as a aromatic heterocyclic ring system (monocyclic or bicyclic) where the heteroaryl moieties are selected from: (1) furan, thiophene, indole, azaindole, oxazole, thiazole, isoxazole, isothiazole, imidazole, N-methylimidazole, pyridine, pyrimidine, pyrazine, pyrrole, N-methylpyrrole, pyrazole, N-methylpyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1-methyl-1,2,4-triazole, 1H-tetrazole, 1-methyltetrazole, benzoxazole, benzothiazole, benzofuran, benzisoxazole,

benzimidazole, N-methylbenzimidazole, azabenzimidazole, indazole, quinazoline, quinoline, and isoquinoline; (2) a bicyclic aromatic heterocycle where a phenyl, pyridine, pyrimidine or pyridizine ring is: (a) fused to a 6-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom; (b) fused to a 5 or 6-membered aromatic (unsaturated) heterocyclic ring having two nitrogen atoms; (c) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom together with either one oxygen or one sulfur atom; or (d) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one heteroatom selected from O, N or S.

If aryl or heteroaryl is 'optionally substituted', one or two of the following are possible substituents: nitro, -aryl, -heteroaryl, alkoxycarbonyl-, -alkoxy, -alkoxy-alkyl, alkyl-O-C2-C4alkyl-O-, -cyano, -halogen, -hydroxy, -N-R_6R_7, -trifluoromethyl, -trifluoromethoxy, arylalkyl, alkylaryl, R_6R_7N-alkyl-, HO-C1-C6-alkyl-, alkoxyalkyl-, alkyl-S-, -SO_2N-R_6R_7, -SO_2NHR_6, -CO_2H, $CONR_6R_7$, aryl-O-, heteroaryl-O-, -$S(O)_s$-aryl (where s = 0 –2), -alkyl-O-alkyl-NR_6R_7, -alkyl-aryl-O-alkylN-R_6R_7, C1-C6alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy-alkyl-O-, R_6R_7N-alkyl-, and -$S(O)_s$-heteroaryl (where s = 0 –2); Preferred substituents for aryl and heteroaryl include: alkyl, halogen, -N-R_6R_7, trifluoromethyl, -trifluoromethoxy, arylalkyl, and alkylaryl.

Arylalkyl is defined as Aryl-C1-C6alkyl---; Arylalkyl moieties include benzyl, 1-phenylethyl, 2-phenylethyl, 3-phenylpropyl, 2-phenylpropyl and the like. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or aryl moiety as defined above.

Alkylaryl is defined as C1-C6alkyl-aryl-. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the aryl or alkyl moiety as defined above.

Heteroaryl-C1-C6- alkyl is defined as a heteroaryl substituted alkyl moiety wherein the alkyl chain is 1-6 carbon atoms (straight or branched). Alkyl heteroaryl moieties include Heteroaryl-$(CH_2)_{1-6}$-- and the like. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or heteroaryl moiety

as defined above;

C1-C6 alkylheteroaryl is defined an alkyl chain of 1-6 carbon atoms (straight or branched) attached to a heteroaryl moiety, which is bonded to the rest of the molecule. Ex. C1-C6-alkyl-Heteroaryl--. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents on the alkyl or heteroaryl moiety as defined above;

Saturated or partially saturated heterocycles groups are defined as heterocyclic rings selected from the moieties; aziridinyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzimidazolyl, dihydrobenzofuranyl, dihydrobenzothienyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidinyl, dihydro-1,4-dioxanyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydroquinolinyl, and tetrahydroisoquinolinyl

C1-C6 alkyl mono or bicyclic saturated or partially saturated heterocycles is defined as an alkyl group (straight or branched) of C1-C6 attached to a heterocycles (which is defined before) through a carbon atom or a nitrogen atom and the other end of the alkyl chain attached to the rest of the molecule. The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or heterocyclic portion of the molecule, as defined before;

Arylalkyloxyalkyl is defined as Aryl-C1-C6alkyl-O-C1-C6alkyl---.The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl and/or aryl portions as defined before;

Alkyloxyalkyl is defined as C1-C6 alkyl-O-C1-C6alkyl---. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at

the alkyl moiety as defined before;

Aryloxyalkyl is defined as Aryl-O-C1-C6 alkyl---. The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl or aryl moiety as defined before;

Heteroarylalkyloxyalkyl is defined as Heteroaryl-C1-C6alkyl-O-C1-C6alkyl---.The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or heteroaryl moiety as defined before;

Aryloxyaryl is defined as Aryl-O-Aryl---.. The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present on the aryl moiety as defined before;

Aryloxyheteroaryl is defined as Aryl-O-Heteroaryl- or –Aryl-O-Heteroaryl; In this definition either the aryl moiety or the heteroaryl moiety can be attached to the remaining portion of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present on the aryl moiety or on the heteroaryl moiety as defined before;

Alkyl aryloxyaryl is defined as Aryl-O-Aryl-C1-C6alkyl----; The term 'optionally substituted' refers to unsubstituted or substituted with1 or 2 substituents present at the aryl moiety as defined before;

Alkylaryloxyheteroaryl is defined as Heteroaryl-O-Aryl-C1-C6alkyl--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the aryl moiety or on the hetroaryl moiety as defined before;

Alkylaryloxyalkylamine is defined as R_6R_7N-C1-C6alkyl-O-Aryl-C1C6alkyl---; The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl or aryl moiety as defined before; R_6 and R_7 as defined before;

Alkoxycarbonyl is defined as C1-C6alkyl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkyl portion of the alkoxy moiety as defined before;

Aryloxycarbonyl is defined as Aryl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the aryl moiety as defined before;

Heteroaryloxy carbonyl is defined as Heteroaryl-O-C=O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Alkoxy is defined as C1-C6alkyl-O--; The terms 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl moiety as defined before;

Aryloxy is defined as Aryl-O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the aryl moiety as defined before;

Heteroaryloxy is defined as Heteroaryl-O--; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Alkenyloxy is defined as C3-C6 alkene-O--; Example allyl-O--, bute-2-ene-O like moieties; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkene moiety as defined before, with the proviso that no hetero atom such as O, S or N-R_1 is present on the carbon atom, which is attached to a double bond;

Alkynyloxy is defined as C3-C6alkyne-O--; Example CH triple bond C-CH_2-O- , like moieties; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyne moiety as defined before, with the proviso

that no hetero atom such as O, S or N-R_1 is present on a carbon atom which is attached to a double or triple bond;

Alkylaminoalkoxy is defined as R_6R_7N-C1-C6-alkyl-O-C1-C6-alkyl---, where the terminal alkyl group attached to the oxygen is connected to the rest of the molecule; The terms R_6 and R_7 are defined above; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl moiety as defined before;

Alkylenedioxy is defined as –O—$(CH_2)_2$---O---;

Aryloxyalkylamine is defined as R_6R_7N-C1-C6-alkyl-O-Aryl--, where the aryl is attached to the rest of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the alkyl or aryl moiety as defined before;

Arylalkenyl is defined as Aryl-C2-C8alkene--, with the proviso that no hetero atom such as O, S or N-R_1 is present on the carbon atom, which is attached to a double bond; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present on the alkene or aryl moiety as defined before;

Heteroaryloxyalkyl is defined as Heteroaryl-O-C1-C6alkyl---; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety as defined before;

Heteroaryloxyaryl is defined as Heteroaryl-O-aryl---, where the aryl moiety is attached to the rest of the molecule; The term 'optionally substituted' refers to unsubstituted or substituted with 1 or 2 substituents present at the heteroaryl moiety or the aryl moiety as defined before;

Alkoxy, alkoxyalkyl, alkoxyalkyloxy and alkylthioalkyloxy are moieties wherein the alkyl chain is 1-6 carbon atoms (straight or branched). Aryloxy, heteroaryloxy, arylthio and heteroarylthio are moieties wherein the aryl and heteroaryl groups are as herein before defined. Arylalkyloxy, heteroarylalkyloxy, arylalkylthio and

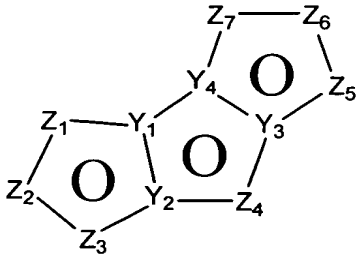
heteroarylalkylthio are moieties wherein the aryl and heteroaryl groups are as herein before defined and wherein the alkyl chain is 1-6 carbons (straight or branched). Aryloxyalkyl, heteroaryloxyalkyl, aryloxyalkyloxy and heteroaryloxyalkyloxy are substituents wherein the alkyl radical is 1-6 carbon atoms. The terms monoalkylamino and dialkylamino refer to moieties with one or two alkyl groups wherein the alkyl chain is 1-6 carbons and the groups may be the same or different. The terms monoalkylaminoalkyl and dialkylaminoalkyl refer to monoalkylamino and dialkylamino moieties with one or two alkyl groups (the same or different) bonded to the nitrogen atom which is attached to an alkyl group of 1-3 carbon atoms.

The expression "Fused tricyclic heteroaryl group" is used in the specification and claims to mean:

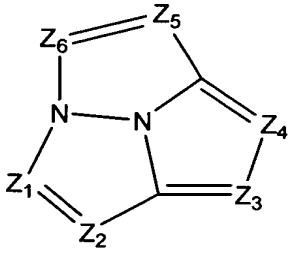
a group comprising three fused rings in which at least one ring has aromatic character (i.e meets Huckel's rule (4n+2)). The fused tricyclic heteroaryl group contains 1-6 heteroatoms selected from the group consisting of O, S, N and N-R_1. The fused tricyclic heteroaryl must be bonded through a carbon preferably in one of the at least one aromatic rings to the remainder of the formula I molecule. The fused tricyclic heteroaryl group may contain 1-3 aromatic rings and 0-2 non-aromatic rings. Each aromatic ring(s) in the fused tricyclic heteroaryl group may contain 5 to 7 ring atoms (including the bridgehead atoms) selected from CR_2, O, S, N, and N-R_1. Each of the aromatic ring(s) of the fused tricyclic heteroaryl group may contain 0 to 3 heteroatoms selected from O, S, N or N-R_1. The non-aromatic ring(s), if any, of the fused tricyclic heteroaryl group may contain 5-8 ring atoms (including bridgehead atoms) and contain 0-4 heteroatoms selected from N, N-R_1, O or $S(O)_n$, wherein n is 0-2. In each non-aromatic ring of the fused tricyclic heteroaryl group, one or two of the non-bridgehead carbon atoms may each be optionally substituted with one or two R_4, and each R_4 may be independently the same or different.

Examples of fused tricyclic heteroaryl groups include:

Ring size and arrangements: **(5-5-5)**



1-A

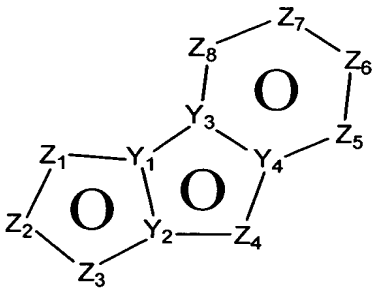


1-B

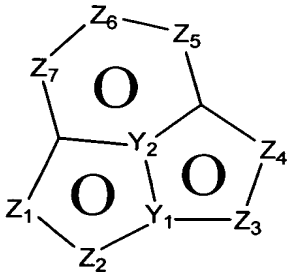
In both formula **1-A** and **1-B** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 and Z_7 are independently selected from CR_2 , N, O, S or N-R_1 and as mentioned above one of Z_1 - Z_7 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 may independently be C or N.

Ring size and arrangement: **(5-5-6)**

2-A

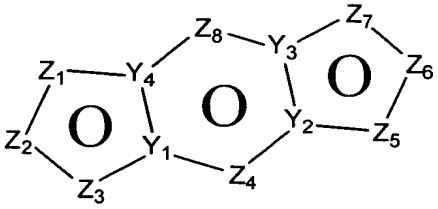


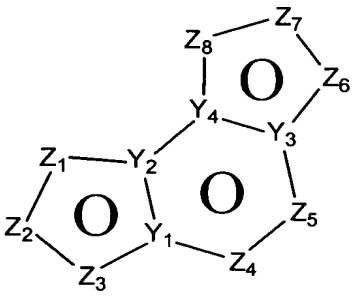
2-B



In both formula **2-A** and **2-B** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 , Z_7 and Z_8 are independently selected from CR_2 , N, O, S or N-R_1 and as mentioned above one of the $Z_1 - Z_8$ is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 may be independently be C or N.

Ring size and arrangement: **(5-6-5)**

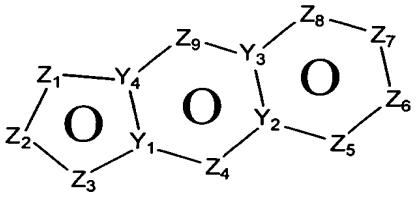


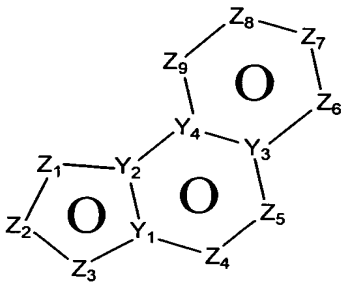


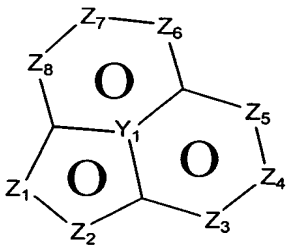
3-A **3-B**

In both formula **3-A** and **3-B** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 , Z_7 and Z_8 are independently selected from CR_2, N, O, S or N-R_1 and as mentioned above one of Z_1 – Z_8 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 may be C or N.

Ring size and arrangements: **(5-6-6)**



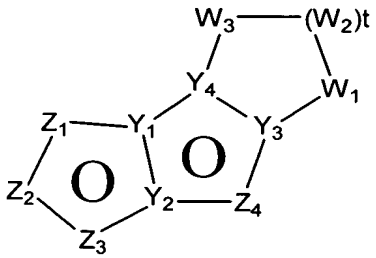




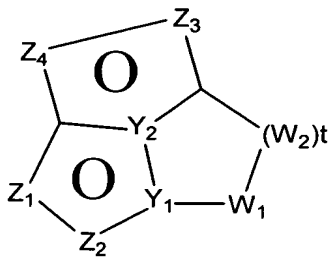
4-A **4-B** **4-C**

In formula **4-A** , **4-B** and **4-C** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 , Z_7 and Z_8 are independently selected from CR_2 , N, O, S or N-R_1 and as mentioned above one of the Z_1 - Z_8 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2, Y_3 and Y_4 are independently C or N.

Ring size and arrangements: **[5-5-(non-aromatic)]**



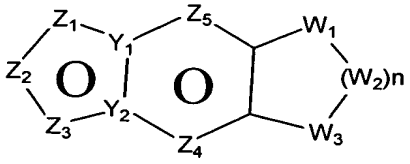
5-A



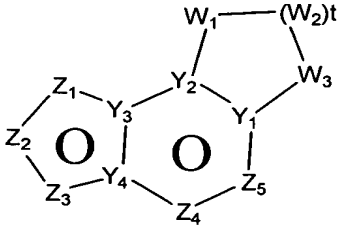
5-B

In both formula **5-A** and **5-B** Z_1 , Z_2, Z_3 and Z_4 are indpendently selected from CR_2, N, O, S or N-R_1 and as mentioned above one of the Z_1 - Z_4 is a carbon atom to which the penem portion of the molecule is attached; Y_1, Y_2 , Y_3 and Y_4 are independently C or N. W_1, W_2 and W_3 are independently selected from CR_4R_4, S(O)r (r = 0 –2) , O, and N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 1 to 3.

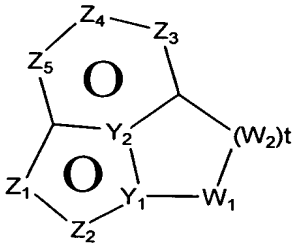
Ring size and arrangement: **[5-6-(non-aromatic)]**



6-A



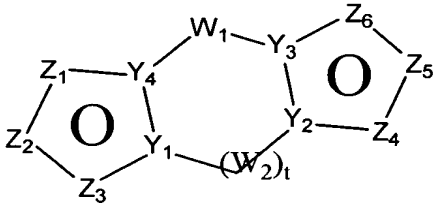
6-B



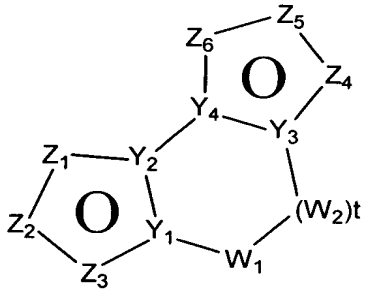
6-C

In formulae **6-A**, **6-B** and **6-C** Z_1 , Z_2, Z_3, Z_4 and Z_5 are independently selected from CR_2, N, O, S or N-R_1 and as mentioned above one of the Z_1 - Z_5 is a carbon atom to which the penem portion of the molecule is attached. Y_1, andY_2 are independently C or N. W_1, W_2 and W_3 are independently selected from CR_4R_4 , S(O)r (r = 0 –2) , O, and N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 1 to 3.

Ring size and arrangement: **[5-(non-aromatic)-5]**



7-A

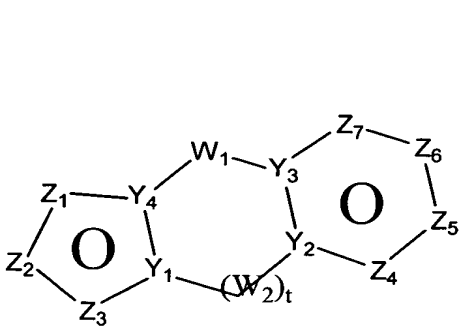


7-B

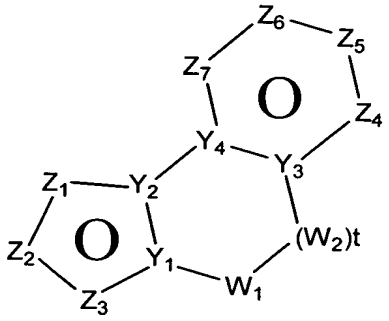
In formulae **7-A** and **7-B** Z_1 , Z_2, Z_3, Z_4, Z_5 and Z_6 are independently selected from CR_2 , N, O, S or N-R_1 and as mentioned above one of Z_1 – Z_6 is a carbon atom to

which the penem portion of the molecule is attached. $Y_{1,}Y_2$, Y_3 and Y_4 are independently C or N. W_1 and W_2 are independently selected from CR_4R_4 , S(O)r (r = 0 –2) , O, and N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 1 to 3.

Ring size and arrangement: **[5-(non-aromatic)-6]**



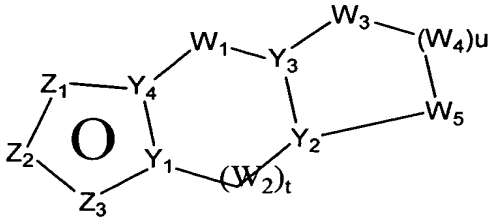
8-A



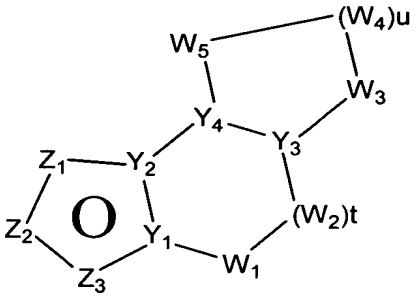
8-B

In formulae **8-A** and **8-B** Z_1 , Z_2, Z_3, Z_4, Z_5, Z_6 and Z_7 are independently selected from CR_2 , N, O, S or N-R_1 and as mentioned above one of the $Z_1 - Z_7$ is a carbon atom to which the penem portion of the molecule is attached. $Y_{1,}Y_2$, Y_3 and Y_4 are independently C or N. W_1 and W_2 are independently CR_4R_4 , S(O)r (r = 0 –2) , O, or N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 0 to 3.

Ring size and arrangement **[5-(non-aromatic)-(non-aromatic)]**



9-A

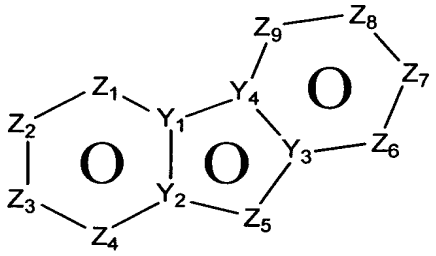


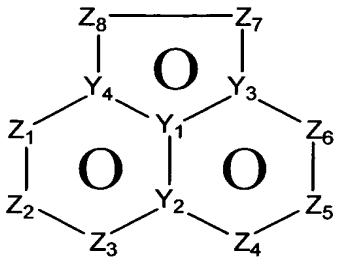
9-B

In formulae **9-A** and **9-B** Z_1 , Z_2 and Z_3 are independently selected from CR_2 N, O, S and N-R_1 and as mentioned above one of the $Z_1 - Z_3$ is a carbon atom to which the penem portion of the molecule is attached. Y_1 and Y_4 are independently C or N; Y_2

and Y_3 are independently CH or N; W_1, W_2 W_3, W_4 and W_5 are independently CR_4R_4, S(O)r (r = 0 –2), O, or N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; t = 0 to 2 and u = 1 to 3.

Ring size and arrangement **(6-5-6)**

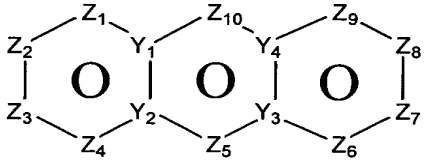


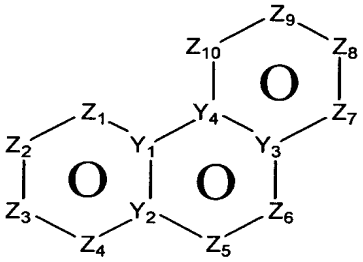


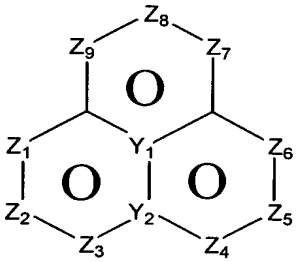
10-A **10-B**

In formula **10-A** and **10-B** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 , Z_7, Z_8 and Z_9 are independently CR_2, N, O, S or N-R_1 and as mentioned above one of the $Z_1 – Z_9$ is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N.

Ring size and arrangement **(6-6-6)**



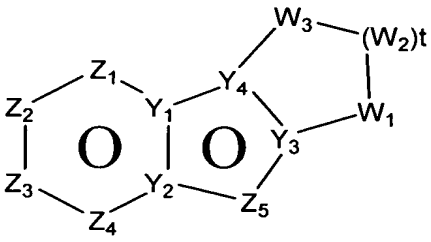


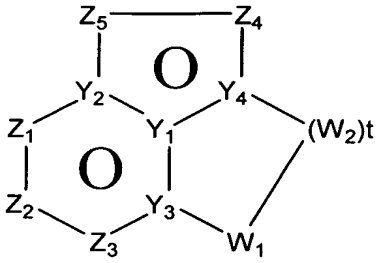


11-A **11-B** **11-C**

In formula **11-A** , **11-B** and **11-C** Z_1 , Z_2, Z_3, Z_4, Z_5 , Z_6 , Z_7, Z_8, Z_9 and Z_{10} are independently CR_2 , N, O, S or N-R_1 and as mentioned above one of $Z_1 – Z_{10}$ is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N.

Ring size and arrangement **[6-5-(non-aromatic)]**

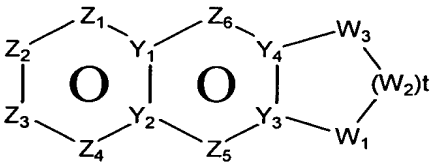


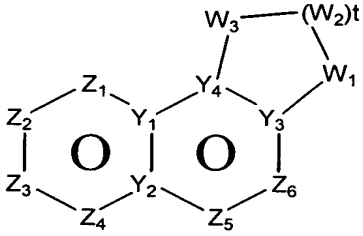


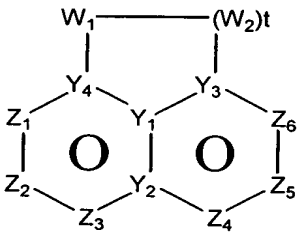
12-A **12-B**

In formula **12-A** and **12-B** Z_1 , Z_2, Z_3, Z_4 and Z_5 are independently CR_2 , N, O, S or N-R_1 with the proviso that one of Z_1 – Z_5 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N; W_1, W_2, W_3 are indepdently O, N-R_1, or S=$(O)_r$ (r = 0-2) with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t =1-4.

Ring size and arrangement **[6-6-(non-aromatic)]**



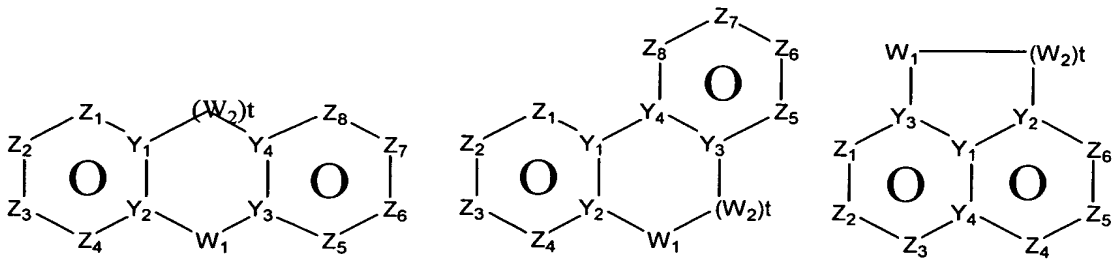




13-A **13-B** **13-C**

In formula **13-A**, **13-B** and **13-C** Z_1 , Z_2, Z_3, Z_4, Z_5 and Z_6 are independently CR_2 , N, O, S or N-R_1 and as mentioned above one of the Z_1 – Z_6 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N; W_1, W_2 and W_3 are independently CR_4R_4 , S(O)r (r = 0 –2) , O, or N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 1 to 3.

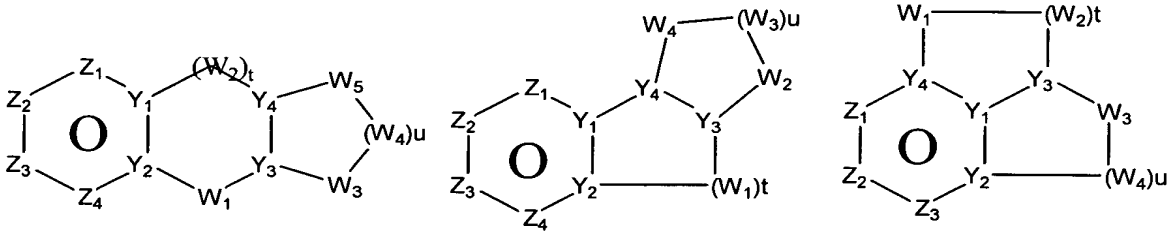
Ring size and arrangement **[6-(non-aromatic)-6]**



14-A **14-B** **14-C**

In formula **14-A**, **14-B** and **14-C** Z_1 , Z_2, Z_3, Z_4, Z_5, Z_6, Z_7 and Z_8 are independently CR_2, N, O, S or N-R_1 and as mentioned above one of Z_1 – Z_8 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N; W_1, W_2 and W_3 are independently CR_4R_4 , S(O)r (r = 0 –2) , O, or N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; and t = 1 to 2.

Ring size and arrangement **[6-(non-aromatic)-(non-aromatic)]**



15-A **15-B** **15-C**

In formula **15-A**, **15-B** and **15-C** Z_1 , Z_2, Z_3 and Z_4 are independently CR_2, N, O, S or N-R_1 and as mentioned above one of Z_1 – Z_4 is a carbon atom to which the penem portion of the molecule is attached. Y_1, Y_2 , Y_3 and Y_4 are independently C or N; W_1, W_2 , W_3 and W_4 are independently CR_4R_4 , S(O)r (r = 0 –2) , O, or N-R_1 with the proviso that no S-S, S-O or O-O bond formation can occur to form a saturated ring; t = 1 to 3 and u = 1 to 3.

The expression 'fused bicyclic heteroaryl group' is used in the specification and claims to mean:

A group comprising two fused rings in which one has aromatic character [i.e. Huckel's rule (4n+2)] and the other ring is non-aromatic;

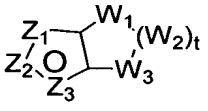
the fused bicyclic heteroaryl group contains one to six heteroatoms selected from the group O, S, N and N-R_1;

the fused bicyclic heteroaryl group is bonded to the remainder of the molecule through a carbon atom in the aromatic ring as shown in the formula **I**;

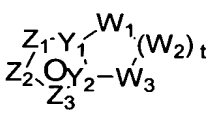
the aromatic ring of the fused bicyclic heteroaryl group contains five or six ring atoms (including bridgehead atoms) selected from CR_2 , N, O, S or N-R_1. The aromatic ring of the fused bicyclic heteroaryl group contains 0 to 3 heteroatoms selected from the group O, S, N and N-R_1;

the non-aromatic ring of the fused bicyclic heteroaryl group contains five to eight ring atoms (including bridgehead atoms) selected from CR_4R_4, N, N-R_1, O, $S(O)_n$ where n= 0-2. The non-aromatic ring of the fused bicyclic heteroaryl group contains 0 to 4 heteroatoms selected from N, N-R_1, O or $S(O)_n$ where n = 0 to 2 .

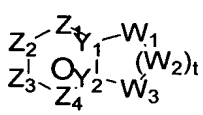
Examples of the fused bicyclic heteroaryl group include:



16-A



16-B



16-C

In formula **16-A** Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 and one of Z1 –Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**. When one of Z's is CR_2 the other two Zs can be either two N or one N and O, S, N-R_1 in any combinations with out disrupting the aromaticity; when two Z,s = CR_2 the other Z can be optionally selected from one N, O, S or N-R_1 in any combination with out disrupting the aromaticity;

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, N-R_1, C=O; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system; t= 1 to 4.

In formula **16-B** Z1, Z2 and Z3 are independently CR_2, N, O, S or N-R_1 and one of Z1 –Z3 is carbon and is bonded to the remainder of the molecule as shown in formula **I**.

When one of Z's = CR_2 , then the other two Z's can be independently CR_2, N, O, S or N-R_1 in any combinations with out disrupting the aromaticity;

When two Z's =N, then the other carbon in the ring is bonded to the penem portion of the molecule as shown in formula **I**.

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, N-R_1,

t= 1 to 4;

Y_1 and Y_2 = N or C; with the proviso that when the aromatic heterocycle is imidazole, the saturated ring may not contain a S adjacent to the bridgehead carbon.

In formula **16-C** Z1, Z2, Z3 and Z4 are independently CR_2 or N and one of Z1 –Z4 is carbon and is bonded to the remainder of the molecule.

W_1, W_2 and W_3 are independently CR_4R_4, S, SO, SO_2, O, or N-R_1; with the proviso that no S-S or O-O or S-O bond formation can occur to form the saturated ring system; t= 1 to 4.

Y_1 and Y_2 are independently C or N.

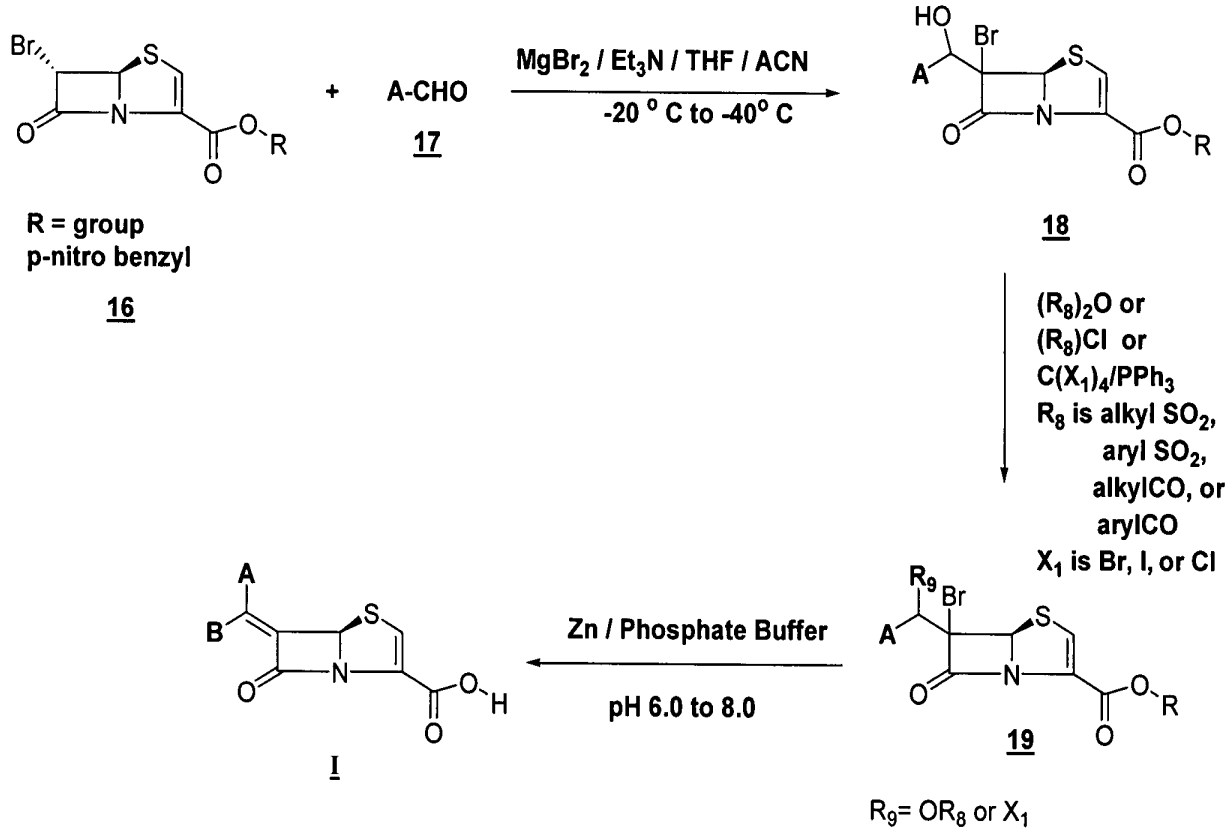
PROCESS OF THE INVENTION

Compounds of the general formula **I** can be prepared in a mild and facile way by condensing an appropriately substituted aldehyde **17** with 6-bromo-penem derivative of structure **16**. (Scheme 2) in the presence of a Lewis acid, preferably anhydrous magnesium halide more preferably anhydrous $MgBr_2$ or $MgBr_2$: etherate and a mild base such as triethylamine, DMAP, or diisopropyl ethyl amine, at low temperature preferably at about —20°C to –40°C. The intermediate aldol product **18** can be functionalized with acid chlorides or anhydrides preferably to an acetate, triflate or a tosylate **19**; or, if formulae **18** is isolated, it can be converted to a halogen derivative by reacting **18** with tetrahalomethane and triphenyl phosphine at room temperature in a suitable organic solvent preferably CH_2Cl_2. The compound **19** can be smoothly converted to the desired product by a reductive elimination process using a metal such as activated zinc and phosphate buffer at mild temperatures preferably about 20°C to 35°C at a pH of about 6.5 to 8.0 or hydrogenating over a catalyst preferably palladium on charcoal. It should be noted that the reductive elimination step could be conducted such that deprotection of the carboxyl group occurs. If the protecting group on the carboxylate oxygen is *para*-nitrobenzyl substituent then the reductive elimination and deprotection can be achieved by a single step. However if the

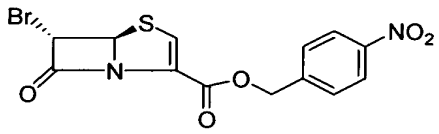
protecting group is other than *para*-nitrobenzyl substituent, a two step procedure can be followed depending up on the nature of the protecting group. The other protecting group include p-methoxy benzyl, benzhydrol, trityl, allyl or alkyl. The product can be isolated as a free acid or as an alkalie metal salt. The above mentioned two step procedure can be carried out in one step by carrying out the entire process without isolating the intermediate **19**. This is a relatively simple procedure and extremely efficient in terms of yield and economic feasibility which can be used to make a wide variety of compounds. This procedure is amenable to a large scale synthesis and applicable to a variety of aldehydes.

The above mentioned aldol condensation reaction is very versatile and it can be applied to any bromopenem derivative, where the carboxy group is protected other than 4-nitrobenzyl moiety. Example of other protecting group include benzyl, para-methoxy benzyl derivative, benzyhydrol , trityl, alkyl and allyl derivatives. However, when the protecting group is other than 4-nitrobenzyl group, a separate deprotection step need to be carried out after the reductive elimination procerdure. The chemistry involved in the deprotection step is well known to people who are skilled in that art.

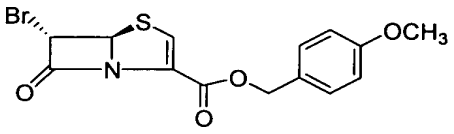
SCHEME 2


Br
S
N
O
O
R
+
A-CHO
17
MgBr2 / Et3N / THF / ACN
-20 ° C to -40° C
HO
Br
A
18
R = group
p-nitro benzyl
16
(R8)2O or
(R8)Cl or
C(X1)4/PPh3
R8 is alkyl SO2,
aryl SO2,
alkylCO, or
arylCO
X1 is Br, I, or Cl
R9
Zn / Phosphate Buffer
pH 6.0 to 8.0
B
H
19
I
R9= OR8 or X1

One important aspect of the present invention relates to the use of stable bromopenem intermediate **16**. The stability of the intermediate is relevant to the aldol condensation step where the decomposition is minimized [as well as to the shelf life stability]. In this invention the key intermediate **16**, where R = *para*-Nitrobenzyl group is a stable and a crystalline intermediate. In the present invention, it has been found that the intermediate **16** is more stable than the intermediate **Q**. The comparative thermal stability data for the intermediates **16** and **Q** are given below. (Table 1) This remarkable stability of intermediate **16** enhances the shelf-life time of the compound as well as the scale-up feasibility. As mentioned above, protection of carboxyl group with *para*-Nitrobenzyl group reduces the number of steps in the present process of preparing the final compound of structure **I**. The crystalline nature of the intermediate was established by X-ray diffraction studies. Table 2 shows the X-ray powder parameters of the formula **16**.



16



Q

Table 1		
Residual Ratio(%)[1]		
Day	**Formula 16**	**Formula Q**
0	100.0	100.0
1	100.0	99.7
2	100.0	95.3
4	100.0	72.9
7	98.7	69.8
14	98.4	Not tested

1) Checked by HPLC analysis.
Temp. 40^{0}C in a solid state.
HPLC conditions:

Column: Inertsil SIL 100-5 4.6X150 mm (GL Science Inc.)

Mobile phase; Ethylacetae: n-hexane (10:35 vv)

Wave length: 312 nM; Flow rate: 1.0 ml/min @ Room temperature.

Table 2

Peak #	2 θ	d value (Q)	intensity (cps)	Relative intensity
1	9.540	9.2631	1575	28
2	13.560	6.5246	1225	22
3	15.460	5.7268	3913	70
4	18.680	4.7462	2509	45
5	19.180	4.6236	820	15
6	19.940	4.4491	1366	25
7	21.060	4.2149	699	13
8	22.360	3.9727	1652	30
9	23.740	3.7448	5560	100
10	24.260	3.6657	3560	64
11	24.940	3.5673	1958	35
12	25.180	3.5338	1799	32
13	25.780	3.4529	878	16
14	26.540	3.3558	1514	27
15	26.900	3.3117	1136	20
16	28.900	3.0869	2206	40
17	29.220	3.0538	1507	27
18.	29.720	3.0035	1473	26
19	30.560	2.9229	906	16
20	31.200	2.8644	815	15
21	31.480	2.8395	888	16
22	34.700	2.5830	906	16
23	36.700	2.4467	785	14
24	37.780	2.3792	862	16
25	38.980	2.3087	762	14

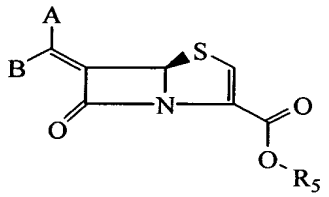
26	40.180	2.2425	728	13
27	41.220	2.1883	718	13

Advantageously, during the reductive elimination step, in the present invention the desired "Z" isomer is formed extremely preferentially (about 50:1) 45-55:1. Formation of the "E" isomer is not observed in the reaction mixture. The reductive elimination step can also be carried out by hydrogenating the intermediate **19** over 10% Pd/C.

The intermediate **16** used in the present invention may be prepared from the commercially available 6-aminopenicillanic acid (6-APA) **20**. (Scheme 3). This may be converted to the bromopenem **16** by the procedure outlined in Scheme 3. 6-aminopenicillanic acid (6-APA) **20** was converted to the bromo-PNB derivative **22** by a one pot procedure. This was oxidized to sulfoxide **23** which under went a ring opening reaction to yield **24.** Compound **24** was converted to **16** by the procedure outlined in Scheme 3. Advantageously, in the present invention, conversion of compound **23** to **26** can be carried out in one pot, without isolating the intermediates **24** and **25**. The bromopenem **16** thus obtained was reacted with the aldehyde **17** (Ref. Scheme 2) in the presence of anhydrous $MgBr_2$ or commercially available $MgBr_2$: $O(Et)_2$. The aldol product was trapped as an acetate and the bromo acetate **19** can be conveniently converted to the final product **I** by reacting it with activated zinc (e.g. freshly activated with 0.1 N HCl) and phosphate buffer (6.5 pH) at room temperature. The product can be conveniently purified by knowns means such as dianion HP-21 column chromatography. Initially the column can be eluted with water to remove any inorganic impurity and latter with 10% MeCN; water. The product obtained through this process is 98% pure and it can be further purified by crystallization. The aldol condensation reaction using Et_3N/ and $MgBr_2$ is extremely efficient and general. The present invention may be extended to a variety of aldehydes to yield the final product of general structure **I**. The above mentioned aldol condensation reaction is very versatile and it can be applied to any bromopenem derivative, where the carboxy group is protected other than 4-nitrobenzyl moiety. Example of other protecting group include benzyl, para-methoxy benzyl derivative, benzhydrol, trityl, alkyl and allyl derivatives. However, when the protecting group is other than 4-nitrobenzyl group, a separate deprotection step need to be carried

out after the reductive elimination procerdure. The chemistry involved in the deprotection step is well known to people who are skilled in that art.

The present invention relates to a process for the preparation of compound of formula I



I

wherein

one of A and B denotes hydrogen and the other is aryl optionally substituted with one or two R_2, heteroaryl optionally substituted with one or two R_2, a fused bicyclic heteroaryl optionally substituted with one or two R_2, fused tricyclic heteroaryl optionally substituted with one or two R_2, cycloalkyl optionally substituted with one or two R_2, alkyl optionally substituted with one or two R_2, alkenyl optionally substituted with one or two R_2, alkynyl optionally substituted with one or two R_2, saturated or partially saturated heteroaryl optionally substituted with one or two R_2;

R_5 is H, an in vivo hydrolyzable ester selected from the group C1 –C6 alkyl, C5 – C6 cycloalkyl, CHR_3OCOC1-C6 or a salt selected from the group consisting of Na, K, and Ca;

R_2 is hydrogen, optionally substituted C1-C6 alkyl, optionally substituted C2-C6 alkenyl having 1 to 2 double bonds, optionally substituted C2-C6 alkynyl having 1 to 2 triple bonds, halogen, cyano, $N\text{-}R_6R_7$, optionally substituted C1-C6 alkoxy, hydroxy; optionally substituted aryl, optionally substituted heteroaryl, $COOR_6$, optionally substituted alkyl aryloxy alkylamines, optionally substituted aryloxy, optionally substituted heteroaryloxy, optionally substituted C3-C6 alkenyloxy, optionally substituted C3 –C6 alkynyloxy, C1-C6 alkylamino-C1-C6 alkoxy, alkylene dioxy, optionally substituted aryloxy-C1-C6 alkyl amine, C1-C6 perfluoro alkyl, $S(O)_q$-optionally substituted C1-C6 akyl, $S(O)_q$- optionally substituted aryl where q is 0, 1 or 2, $CONR_6R_7$, guanidino or cyclic guanidino, optionally substituted C1-C6 alkylaryl, optionally substituted arylalkyl, optionally substituted C1-C6

alkylheteroaryl, optionally substituted heteroaryl-C1-C6 alkyl, optionally substituted C1-C6 alkyl mono or bicyclic saturated heterocycles, optionally substituted arylalkenyl of 8 to 16 carbon atoms, $SO_2NR_6R_7$, optionally substituted arylalkyloxyalkyl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted aryloxyaryl, optionally substituted aryloxyheteroaryl, substituted heteroaryloxyaryl, optionally substituted C1-C6alkyl aryloxyaryl, optionally substituted C1-C6 alkylaryloxyheteroaryl, optionally substituted aryloxyalkyl, optionally substituted heteroaryloxyalkyl, optionally substituted alkylaryloxyalkylamines. Preferred R_2 groups are H, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted heteroaryl, halogen, CN, hydroxy, optionally substituted heterocycle, $-CONR_6R_7$, $COOR_6$, optionally substituted aryl, $S(O)_q$-alkyl, and $S(O)_q$-aryl.

R_3 is hydrogen, C1-C6 alkyl, C3 – C6 cycloalkyl, optionally substituted aryl, optionally substituted heteroaryl;

R_6 and R_7 are independently H, optionally substituted C1-C6 alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted C1-C6 alkyl aryl, optionally substituted arylalkyl, optionally substituted heteroarylalkyl, optionally substituted C1-C6 alkyl heteroaryl, R_6 and R_7 can be taken together to form a 3-7 membered saturated ring system optionally having one or two heteroatoms such as $N-R_1$, O, $S=(O)_n$ n = 0-2;

the process for the preparation of compound of formula **I** comprises

Step 1: 6-aminopenicillanic acid **20** dissolved in an organic solvent (preferably methanol or THF) and water was converted to the 6-bromo derivative in the presence of 48% w/w hydrobromic acid at –10° C to –30°C and sodium or potassium nitrite solution. The 6-bromopenicillanic acid **21** derivative either can be isolated or *insitu* converted to the p-Nitrobenzyl 6-brompenicillanate **22** using 4-nitrobenzylbromide in the presence of organic bases or inorganic bases (preferably sodium or potassium carbonate) in an organic solvent. (Preferably THF or DMF).

Step 2: The product 4-nitrobenzyl 6-bromopenicillanate **22** obtained by the process outlined in step 1 can be isolated or be transformed to 4-nitrobenzyl 6-

bromopenicillanate 1-oxide **23** in the same pot (i.e Step 1; sequential formation) by oxidizing **22** to 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** using oxidizing agents such as 3-chloroperoxybenzoic acid (mcpba) or hydrogen peroxide.

Step 3: The product from step 2, namely 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** can be converted to 4-nitrobenzyl(2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-3-enoate **24** by refluxing 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** with 2-mercaptobenzothiazole (HSBT) in an aromatic solvent. (preferably Toluene).

Step 4: The product from step 3, namely 4-nitrobenzyl(2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-3-enoate **24** can be dissolved in an organic solvent (preferably Toluene) and upon reaction with an organic tertiary base (preferably triethylamine) at ambient temperature gave 4-nitrobenzyl-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-2-enoate **25**.

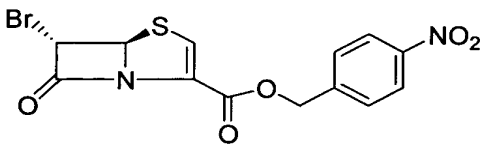
Step 5: The product from step 4, namely 4-nitrobenzyl-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidine-1-yl]-3-methylbut-2-enoate **25** can be converted to **26** 4-nitrobenzyl 2-[(3S,4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate by carrying out the reaction in an aromatic organic solvent (Preferably Toluene) in the presence of an organic acid (Preferably formic acid) , acetic anhydride/ organic tertiary base (preferably Pyridine) and trialkyl or triaryl phosphine (preferably triphenylphosphine) at –10° C to –30° C.

Sequential conversion of compound 23 to 26 with out isolating the intermediates:

Product from Step 2 namely 4-nitrobenzyl 6-bromopenicillanate 1-oxide **23** was reacted with mercaptobenzothiazole in refluxing aromatic organic solvent (preferably Toluene) for 1 to 3 hrs and treated with triethylamine at 0 to –20° C for 3 to 4 hrs. After this treatment, reaction mixture was charged with organic acid (preferably formic acid) and an anhydride (acetic anhydride), an organic tertiary base (preferably pyridine) and a trialkyl or triaryl phosphate sequentially at –10° C to -40° C.

Step 6: The product 4-nitrobenzyl 2-[(3S,4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate **26** from either step 5 or from the sequential conversion was taken up in an organic solvent (preferentially ethyl acetate) at –70°C to –90° C and ozonized oxygen was passed through it for 3 to 4 hrs followed by intramolecular cyclization using a phosphite reagent (preferably trimethyl phosphite). The product 4-nitrobenzyl (5R,6S)-6-bromopenem-3-carboxylate **16** was crystallized from ethylacetae:hexane.

The compound represented by the follwing formula (**16**) :



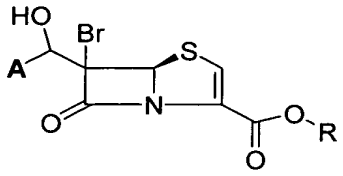
16

An above mentioned process (Step 1 to Step 6) for the preparation of the compound represented by the formula **16** is an intermediate useful for the preparation of 6-(substituted methylene)penems of general formula **I**.

The compound represented by the formula **16** is a crystalline derivative (X-ray powder diffraction parameters are given in the Table 2)

The crystalline nature of this intermediate impart stability and there by increases the shelf-life time. The stability data of the compound of the formula **16** is given in Table 1.

Step 7: Reaction of 4-nitrobenzyl (5R,6S)-6-bromopenem-3-carboxylate **16** with the appropriately substituted aldehydes (defind as before) to effect the aldol condensation step can be carried out in the presence of a Lewis acid (preferably anhydrous $MgBr_2$ or $MgBr_2$:



18

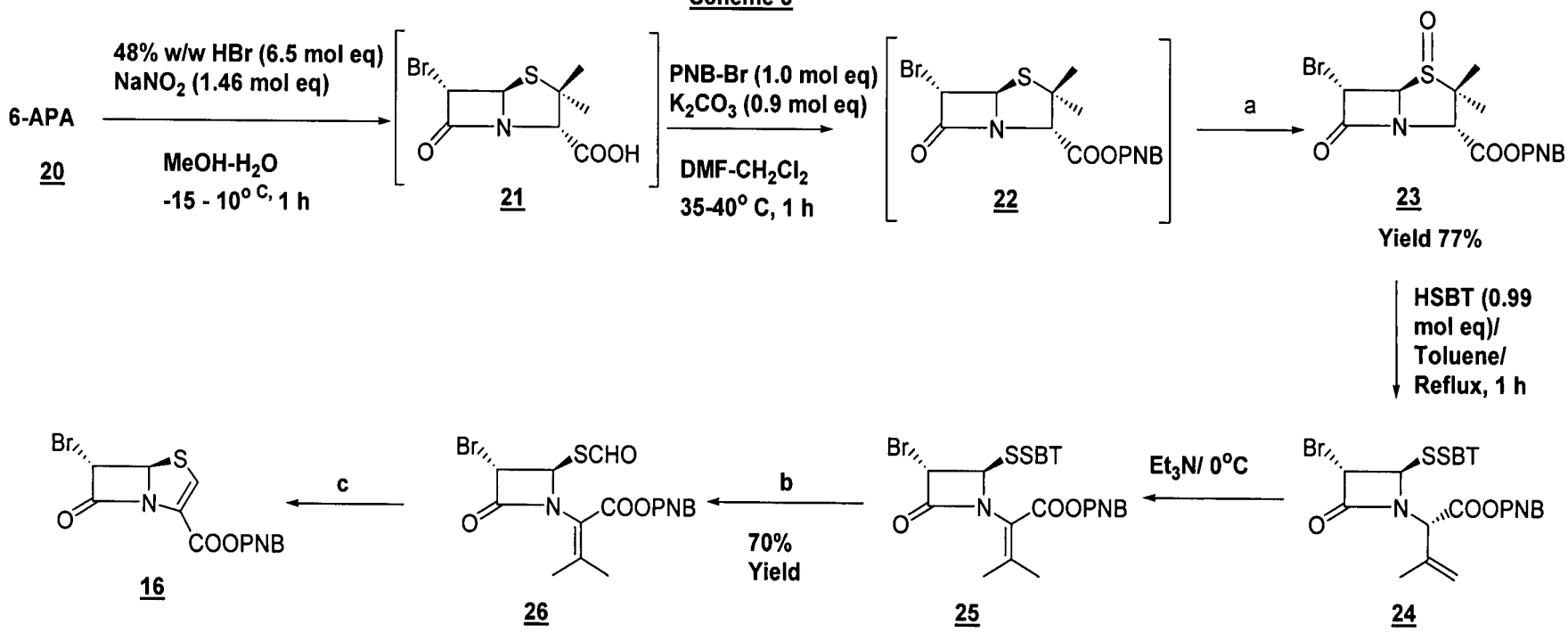
etherate) and a organic tertiary base (preferably triethylamine, DMAP or diisopropyl ethylamine) in an aprotic polar organic solvents (preferably THF and acetonitrile) at temperature –20° C to –40°C. The intermediate aldol products of general formula **18** can be functionalized with an acid chloride or anhydride to an acetate, triflic anhydride to a triflate or tosylchloride to a tosylate at 0°C to – 10° C in the same pot; or, if formulae **18** is isolated, it can be converted to a halogen derivative by reacting **18** with tetrahalomethane and triphenyl phosphine at room temperature in a suitable organic solvent preferably CH_2Cl_2.

Step 8: The final step, namely the reductive elimination step to yield the compound of the general formula **I** can be carried out by dissolving the aldol product mixture in an organic solvent (preferably THF/ acetonitrile) and 6.5 –7.0 pH phosphate buffer and activated metal such as zinc, tin or aluminum at ambient temperature. (preferably at 20° C to 35°C) The product as a alkalie metal salt can be purified by a reverse phase resin column chromatography.

Alternatively Step 7 and Step 8 can be carried out sequentially in the same pot with out isolating the aldol intermediate.

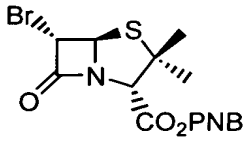
The final step, namely the reductive elimination step can also be carried out by dissolving the aldol product in an organic solvent and 6.5-7.0 phosphate buffer and hydrogenating over Pd/C at 10 to 100 psi (preferably at 40 psi) pressure.

Scheme 3



PNB= *para*-Nitrobenzyl. a: mcpba (0,89 eq)/ DMF-CH_2Cl_2/ 0° C, 20 min. Crystallization; b: (1)HCOOH (4.4 mol eq)/ AC_2O (4.4 mol eq)/ Pyridine (1.03 mol eq)/ MeCN, -15°C, 5 min; (2) Ph_3P (1.02 mol eq) -15°C, 1 h. (3) Column; (4) Crystallization; c: (1) O_3, AcOEt, -70° C, 1.75 hrs; (2) $P(OMe)_3$ (4.8 mol eq), Reflux, 45 min; (3) Crystallization

Experimentals



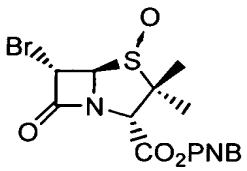
Example 1: Preparation of Sodium (5R)-(Z)-6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-carboxylate

Step 1: p-Nitrobenzyl 6-bromopenicillanate

6-Aminopenicillanic acid (5.0 g) was added to the cooled solution of methanol (44 mL), water (14 mL), and 48% w/w hydrobromic acid (14 mL) at -10 °C. After the addition was complete, the mixture was cooled to –15 °C. Sodium nitrite solution (2.4 g dissolved in 6.6 mL of water) was added over 5 min, and the resulting solution was stirred without cooling for a further 30 min. Sodium chloride (2.4 g) was dissolved in the reaction solution. The reaction mixture was extracted with dichloromethane (2 x 36 mL). The combined organic layers were washed with brine (36 mL), dried ($MgSO_4$), and concentrated to 20 mL under reduced pressure at 25 °C. The residual solution contains 6-bromopenicillanic acid and used to next reaction as it is.

Anhydrous potassium carbonate (2.9 g), dimethylformamide (40 mL), and 4-Nitrobenzyl bromide (5.0 g) were successively added to the residual solution and the mixture was stirred at 35 - 40 °C for 1 h. The reaction solution was poured into a mixture of water (33 mL) and dichloromethane (41 mL) and the organic layer was separated. The organic layer was washed with water (40 mL) and brine (40 mL), dried ($MgSO_4$), and evaporated. The residue was applied to silica gel column chromatography, eluted with ethyl acetate - hexane (4/1), and the title compound was obtained as a colorless solid (7.2 g, 75%).

^{1}H-NMR (δ, CDCl3): 1.41 (s, 3H), 1.62 (s, 3H), 4.61 (s, 1H), 4.83 (d, 1H, J = 1.5 Hz), 5.25 and 5.34 (AB, 2H, J = 13.0 Hz), 5.41 (d, 1H, J = 1.5 Hz), 7.56 (d, 2H, J = 8.7 Hz), 8.26 (d, 2H, J = 8.7 Hz).



Step 2: p-Nitrobenzyl 6-bromopenicillanat 1-oxide

p-Nitrobenzyl 6-bromopenicillanate (1.36 g) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. 3-Chloroperbezoic acid (0.56 g) was added to the solution and stirred for 10 min. The reaction solution was washed with saturated sodium hydrogen carbonate, water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate - hexane (1/1), and the title compound was obtained as a colorless solid (1.4 g, quantitative).

^{1}H-NMR (δ, CDCl3): 1.17 (s, 3H), 1.69 (s, 3H), 4.60 (s, 1H), 5.04 (d, 1H, J = 1.5 Hz), 5.10 (d, 1H, J = 1.5 Hz), 5.30 and 5.37 (AB, 2H, J = 12.9 Hz), 7.56 (d, 2H, J = 8.7 Hz), 8.27 (d, 2H, J = 8.7 Hz).

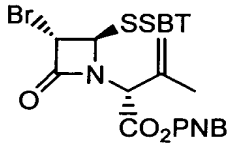
Sequential Preparation of p-Nitrobenzyl 6-bromopenicillanate 1-oxide:

6-Aminopenicillanic acid (500 g) was added to the cooled solution of methanol (3.5 L), water (1.36 L), and 48% w/w hydrobromic acid (1.36 L) at -10 °C. After the addition was complete, the mixture was cooled to –15 °C. Sodium nitrite solution (235 g dissolved in 660 mL of water) was added over 30 min, and the resulting solution was stirred without cooling for a further 30 min. Sodium chloride (240 g) was dissolved in the reaction solution. The reaction mixture was extracted with dichloromethane (2 x 3 L). The combined organic layers were washed with brine (3 L), dried ($MgSO_4$), and concentrated to 1.6 L under reduced pressure at 25 °C. The residual solution contains 6-bromopenicillanic acid and used to next reaction as it is.

Anhydrous potassium carbonate (288 g), dimethylformamide (2.8 L), and 4-Nitrobenzyl bromide (500 g) were successively added to the residual solution and the mixture was stirred at 35 - 40 °C for 1 h. The reaction solution was poured into a mixture of water (2.8 L) and dichloromethane (3.4 L) and the organic layer was separated. The organic layer was washed with water (2.8 L) and brine (3 L) and cooled to –2 °C. The organic layer contains p-nitrobenzyl 6-bromopenicillanate and used to next reaction as it is.

3-Chloroperbezoic acid (355 g) was added to the organic layer over 15 min and stirred for 10 min. The reaction mixture was diluted with ethyl acetate (4.8 L) and washed with saturated sodium hydrogen carbonate (7.6 L). The organic layer was

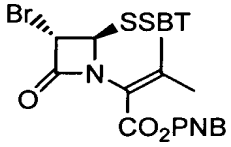
separated and the aqueous layer was re-extracted with ethyl acetate (3.9 L). The combined organic layers were washed with water (3.9 L) and brine (3.9 L), dried ($MgSO_4$), and evaporated under reduced pressure at 30 °C. The residual solid was triturated with ethyl acetate (1.8 L) for 30 min and hexane (5.4 L) was added dropwise to this mixture over 1 h. The solid was filtered off and the filter cake was washed with ethyl acetate – hexane (1/4, 0.8 L). The solid was air-dried for 1 h and dried in vacuo overnight at room temperature. The title compound was obtained as a pale-yellow crystalline solid (768 g, 77%).



Step 3: p-Nitrobenzyl (2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidin-1-yl]-3-methylbut-3-enoate

p-Nitrobenzyl 6-bromopenicillanate 1-oxide (4.31 g) and mercaptobenzothiazole (1.67 g) were heated in refluxing toluene (14 mL) with provision for the azeotropic removal of water (Dean & Stark apparatus) for 1 h. After cooling to room temperature, the reaction solution was evaporated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate – hexane (2/5), and the title compound was obtained as a pale yellow solid (5.67 g, 98%).

^{1}H-NMR (δ, CDCl3): 1.92 (s, 3H), 4.87 (s, 1H), 5.05 (s, 1H), 5.10 (d, 1H, J = 1.7 Hz), 5.21 (s, 4H), 7.39 (t, 1H, J = 7.1 Hz), 7.41 – 7.50 (m, 3H), 7.81 (d, 1H, J = 8.0 Hz), 7.90 (d, 1H, J = 8.0 Hz), 8.20 (d, 2H, J = 8.8 Hz).



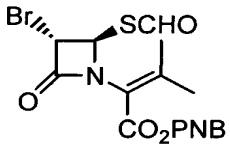
<u>Step 4:</u> p-Nitrobenzyl 2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidin-1-yl]-3-methylbut-2-enoate

p-Nitrobenzyl (2R)-2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidin-1-yl]-3-methylbut-3-enoate (5.1 g) was dissolved in toluene (50 mL), cooled to 0 °C, and treated with triethylamine (0.12 mL). After being stirred at 0 °C for 2 h, the reaction

solution was washed with 1M HCl, water, saturated sodium hydrogen carbonate, and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate – hexane (1/3), and the title compound was obtained as a colorless solid (4.16 g, 81%). The more polar fraction contains the symmetric disulfide as a colorless solid (0.68 g, 19%).

^{1}H-NMR (δ, CDCl3): 2.01 (s, 3H), 2.24 (s, 3H), 5.01 (d, 1H, *J* = 1.7 Hz), 5.24 and 5.30 (AB, 2H, *J* = 13.1 Hz), 5.35 (d, 1H, *J* = 1.7 Hz), 7.34–7.42 (m, 1H), 7.48 –7.57 (m, 1H), 7.80 (d, 1H, *J* = 8.0 Hz), 7.90 (d, 1H, *J* = 8.0 Hz), 8.14 (d, 3H, *J* = 8.7 Hz).

Symmetric disulfide ; ^{1}H-NMR (δ, CDCl3): 2.01 (s, 6H), 2.29 (s, 6H), 4.73 (d, 2H, *J* = 1.7 Hz), 5.06 (d, 2H, *J* = 1.7 Hz), 5.32 (s, 4H), 7.54 (d, 4H, *J* = 8.6 Hz), 8.24 (d, 4H, *J* = 8.6 Hz).



Step 5:p-Nitrobenzyl 2-[(3S, 4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate

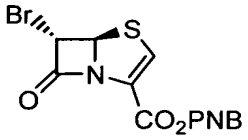
p-Nitrobenzyl 2-[(3S,4R)-4-(benzothiazol-2-yldithio)-3-bromo-2-oxoazetidin-1-yl]-3-methylbut-2-enoate (3.16 g) was suspended in toluene (20 mL) and cooled to –20 °C. Formic acid (1.10 g), acetic anhydride (2.45 g), and pyridine (0.44 g) were added sequentially. The mixture was cooled to –20 °C. Triphenylphosphine (1.45 g) was added in portions over 5 min. After being stirred at –15 ~ –10 °C for further 1 h, the resulting suspension was cooled to –30 °C then filtered. The residual solid was rinsed with cold (-30 °C) toluene (15 mL). The combined filtrate was washed successively with a mixture of ice-water (8 mL) and ice-cold brine (1 mL), a mixture of ice-water (7 mL) and ice-cold brine (3 mL), cold saturated sodium hydrogen carbonate (2 x 11 mL), and cold brine (11 mL). The organic layer was dried ($MgSO_4$) and evaporated. The residue was applied to silica gel column chromatography, eluted with ethyl acetate - hexane (1/2), and the title compound was crystallized from a cold isopropanol (10 mL). The solid was filtered off, washed with a cold isopropanol (6 mL), and dried in vacuo at room temperature overnight. The title compound was obtained as a colorless crystalline solid (1.87 g, 77%)

m.p. 104-106 °C; ^{1}H-NMR (δ, CDCl3): 2.00 (s, 3H), 2.30 (s, 3H), 4.82 (d, 1H, *J*

= 1.9 Hz), 5.32 and 5.38 (AB, 2H, J = 13.3 Hz), 5.81 (d, 1H, J = 1.9 Hz), 7.61 (d, 2H, J = 8.6 Hz), 8.25 (d, 2H, J = 8.6 Hz), 10.09 (s, 1H).

Sequential Preparation of p-Nitrobenzyl 2-[(3S, 4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate from p-Nitrobenzyl 6-bromopenicillanate 1-oxide

p-Nitrobenzyl 6-bromopenicillanate 1-oxide (766 g) and mercaptobenzothiazole (294 g) were heated in refluxing toluene (2.4 L) with provision for the azeotropic removal of water (Dean & Stark apparatus) for 1 h. The reaction mixture was cooled to 0 °C and treated with triethylamine (25 mL). After being stirred at 0 °C for 2 h, the resulting suspension was cooled to –20 °C. Formic acid (360 g), acetic anhydride (798 g), and pyridine (145 g) were added sequentially maintaining the temperature below –10 °C. The mixture was cooled to –20 °C. Triphenylphosphine (473 g) was added in portions over 10 min maintaining the temperature between –15 °C and –10 °C. After being stirred at –15 ~ –10 °C for further 1 h, the resulting suspension was cooled to –30 °C then filtered. The residual solid was rinsed with cold (-30 °C) toluene (500 mL). The combined filtrate was washed successively with a mixture of ice-water (2 L) and ice-cold brine (0.26 L), a mixture of ice-water (1.72 L) and ice-cold brine (0.8 L), cold saturated sodium hydrogen carbonate (2 x 2.6 L), and cold brine (2.6 L). The organic layer was dried ($MgSO_4$) and evaporated. The residue was applied to silica gel (6 Kg) column chromatography, eluted with ethyl acetate - hexane (1/2), and the title compound was crystallized from a cold isopropanol (800 mL). The solid was filtered off, washed with a cold isopropanol (400 mL), air-dried for 1 h, and dried over P_2O_5 in vacuo at room temperature overnight. The title compound was obtained as a reddish yellow crystalline solid (553 g, 70%)

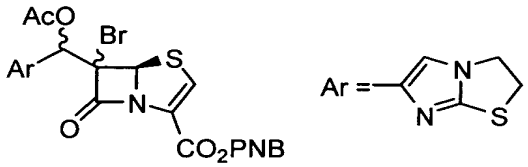


<u>Step 6:</u> p-Nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate

The p-Nitrobenzyl 2-[(3S, 4R)-3-bromo-4-formylthio-2-oxoazetidin-1-yl]-3-methylbut-2-enoate (300 g) was dissolved in ethyl acetate (7.5 L) and cooled to –70 °C.

Ozonized oxygen was passed through the vigorously stirred solution for ca. 1.5 h until a blue color persisted. The solution was purged with nitrogen for 2.5 h, and treated with trimethyl phosphite (384 mL) at –70 °C. The mixture was warmed slowly and stirred at ambient temperature for 17 h. The solution was then heated to reflux gently for 45 min. After cooling to room temperature, the mixture was diluted with ethyl acetate (1.25 L) and washed with water (2 x 2.5 L) and brine (2.5 L). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure at 25 °C. The residual solid was triturated with ethyl acetate (0.6 L). Hexane (0.45 L) was added dropwise to this mixture over 50 min. The solid was filtered off and the filter cake was washed with ethyl acetate – hexane (1/1, 2 x 200 mL) and with ethyl acetate – hexane (1/2, 300 mL). The solid was air-dried for 1 h and dried in vacuo overnight at room temperature. The title compound (109.6 g, 42%) was obtained as a colorless crystalline solid.

m.p. 148-151°C; ^{1}H-NMR (δ, CDCl3): 5.20 (d, 1H, J = 0.9 Hz), 5.29 and 5.43 (AB, 2H, J = 13.5 Hz), 5.81 (d, 1H, J = 1.5 Hz), 7.37 (d, 1H, J = 0.9 Hz), 7.60 (d, 2H, J = 8.7 Hz), 8.25 (d, 2H, J = 8.7 Hz).



Step 7: p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate by using Ph_2NLi and $MgBr_2$ etherate

$MgBr_2$ etherate (335 mg) was added to the dry THF (12 mL) solution of p-Nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (385 mg) under an argon atmosphere at room temperature. The mixture was cooled to –78 °C. The reaction vessel was covered with foil to exclude light. Lithium diphenylamide, prepared by adding n-butyl lithium (0.87 mL, 1.5 mol/L in n-hexane) to a dry THF solution (4 mL) of diphenylamine (220 mg) at –20 °C, was added to the vigorously stirred suspension in one portion. After 15-20 s, the vigorously stirred mixture was treated, in one portion, with a dry acetonitrile solution (5 mL) of 2,3-dihydro-imidazo[2,1-b]thiazole-6-carbaldehyde (170 mg). The reaction mixture was stirred for 1 h at –78 °C and treated with acetic anhydride (133 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 1 h at 0 °C. The mixture was diluted with ethyl acetate and washed with water and brine. The organic

layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate - hexane (1/2 ~ 1/1), and the title compound was obtained as two diastereo mixture (3/1, a colorless amorphous solid, 447 mg, 77%).

Main diastereomer: ^{1}H NMR (δ, $CDCl_3$): 2.00 (s, 3H), 3.76-3.86 (m, 2H), 4.10-4.21 (m, 2H), 5.27 and 5.45 (AB, 2H, J = 13.6 Hz), 6.23 (s, 1H), 6.30 (s, 1H), 7.15 (s, 1H), 7.48 (s, 1H), 7.59-7.65 (m, 2H), 8.24 (d, 2H, J = 8.6 Hz).

Minor diastereomer: ^{1}H NMR (δ, $CDCl_3$): 2.24 (s, 3H), 3.76-3.86 (m, 2H), 4.10-4.21 (m, 2H), 5.27 and 5.45 (AB, 2H, J = 13.6 Hz), 6.08 (s, 1H), 6.79 (s, 1H), 6.91 (s, 1H), 7.44 (s, 1H), 7.59-7.65 (m, 2H), 8.24 (d, 2H, J = 8.6 Hz).

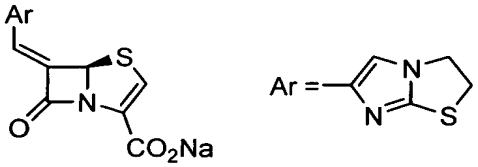
Preparation of p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate by using by using anhydrous MgBr2 and Et3N

The 2,3-dihydro-imidazo[2,1-b]thiazole-6-carbaldehyde (88 mg) was added to the dry acetonitrile (4 mL) solution of anhydrous $MgBr_2$ (115 mg) under an argon atmosphere at room temperature. Colorless powder was deposited over 30 min. The dry THF solution (4 mL) of p-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (200 mg) was added, cooled to –20 °C, and triethylamine (0.18 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (0.99 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate - hexane (1/2 ~ 1/1), and the title compound was obtained as two diastereo mixture (10/1, a colorless amorphous solid, 286 mg, 90%).

Preparation of p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate by using $MgBr_2$ etherate and Et_3N

$MgBr_2$ etherate was used for the reaction instead of anhydrous $MgBr_2$ and the

product was isolated in 84% yields.



Step 8: Sodium (5R)-(Z)-6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-carboxylate

p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate (500 mg) was dissolved with THF (7.0 mL) and acetonitrile (3.2 mL). Freshly activated Zn dust (2.0 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 10.2 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The mixture was diluted with water, cooled to 3 °C, and 1 *M* NaOH was added to adjust pH to 8. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer, contains 224 mg (79%) of the title compound from HPLC assay, was concentrated under high vacuum at 35 °C. The concentrate was applied to Daiaion HP-21 (20 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After absorbing, the column was eluted with water and then with 10% acetonitrile-water to give the purified active fractions of the title compound. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (213 mg, 75%). The amorphous solid of the product was dissolved with water (1.9 mL). Acetone (8 mL) was added to the solution with stirring at room temperature and cooled to 3 °C then stirred for 30 min. Acetone was added to the mixture in 2 mL portions every 30 min over 4 h period at 3 °C (total 16 mL of acetone was added). After stirring for 1 hour at 3 °C, the solid was filtered off and the filter cake was washed with acetone (2 mL). The solid was air-dried under excluding light for 1 h and dried in vacuo overnight at room temperature to give the title compound (200 mg, 71%) as a yellow crystalline solid.

^{1}H NMR (δ, D_2O) 3.82 (d, 2H, J = 7.4 Hz), 4.14-4.20 (m, 2H), 6.42 (s, 1H), 6.82 (s, 1H), 6.96 (s, 1H), 7.42 (s, 1H).

Sequential Preparation of Sodium (5R)-(Z)-6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-carboxylate from p-Nitrob nzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate

The p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate (110.1 g) was added to the dry acetonitrile (5 L) solution of anhydrous $MgBr_2$ (143.4 g) under an argon atmosphere at room temperature. Colorless powder was deposited over 30 min. The dry THF solution (5 L) of p-nitrobenzyl 6-bromopenicillanate 1-oxide (250 g) was transferred to the suspension via a PTFE tube under positive argon pressure over 12 minutes. After the reaction mixture was cooled to –20 °C, triethylamine (217 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (133 g) in one portion. The reaction mixture was warmed to 0°C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate (20 L) and washed with 5% citric acid (10 L) aqueous solution, saturated sodium hydrogen carbonate (10 L), and brine (10 L). The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate (1.4 L). The filtrate was concentrated under reduced pressure at 30 °C. The residual brown oil contains 327 g (86.7%) of the aldol product from HPLC assay.

The residual brown oil of the product was dissolved with THF (5.4 L) and acetonitrile (2.5 L). Freshly activated Zn dust (1.5 Kg) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 7.9 L). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h maintaining the temperature about 35 °C. The mixture was cooled to 3 °C and an ice cold 1 *M* NaOH (ca. 1750 mL) was added to adjust pH to 8 at below 3°C. The mixture was diluted with ethyl acetate (4 L) and filtered through a pad of Celite. The pad was washed with water (2.5 L) and the aqueous layer was separated. The aqueous layer, contains 137 g (64%) of from HPLC assay, was concentrated to 8.85 Kg (ca. 8 L) under high vacuum at 35 °C.

The concentrate was applied to Sepabeads SP-207 (7 L, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After absorbing, the column was eluted with water (14 L) and then with 20% acetonitrile-water to give the purified active fractions of sodium (5R)-(Z)-6-(2,3-dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-carboxylate.

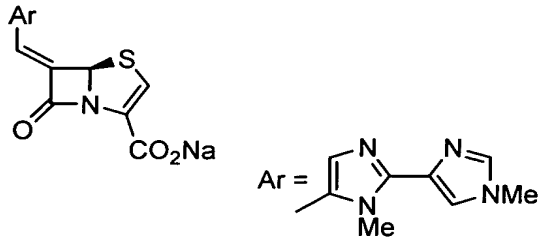
The combined fractions was concentrated to 1.25 Kg (ca. 1 L, contains 130 g (61%) of sodium (5R)-(Z)-6-(2,3-dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-

carboxylate from HPLC assay) under high vacuum at 35°C. Acetone (2.5 L) was added to the concentrate with stirring at room temperature. The mixture was cooled to 3 °C, then stirred for 30 min. Acetone was added to the mixture in 625 mL portions every 30 min over 4 h period at 3 °C (total 5 L of acetone was added). After stirring for 1 hour at 3 °C, the solid was filtered off and the filter cake was washed with acetone (800 mL). The solid was air-dried under excluding light for 1 h and dried in vacuo overnight at room temperature to give the title (112 g, 50% from p-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate) as a yellow crystalline solid.

Sodium (5R)-(Z)-6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)penem-3-carboxylate from 4-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate using hydrogenolysis procedure:

4-Nitrobenzyl (5R)-6-[acetoxy-(2,3-dihydro-imidazo[2,1-*b*]thiazol-6-yl)-methyl]-6-bromopenem-3-carboxylate (116 mg0 and 10% Pd?C (50% wet, 58 mg) were addedto a mixture of THF (4 ml) and 0.5 mol/L phosphate buffer (pH 6.5, 4 ml). The mixture was hydrogenated under H_2 at 40 psi pressure at room temperature for 1 hr. The reaction mixture was filtered, cooled to 3° C and 1M NaOH was added to adjust the pH to 8.0. The mixture was filtered again and the filtrate was washed with ethyl acetate. The combined aqueous layer was concentrated under reduced pressure at 35° C and applied to Diaion HP-21 resin (7 ml, Mitsubishi Kasei Co. Ltd) column chromatography. After adsorbing the column was eluted with water and then 10% acetonitrile; water. The combined pure fractions were concentrated under reduced pressure at 35°C and lyophilized to give the title compound as a yellow amorphous solid.

Example 2



Step 1: 3,1'-Dimethyl-3H,1'H-[2,4']biimidazolyl-4-carbaldehyde

Potassium tert-butoxide (3.23 g) was added to the mixture of dry DMF (140 mL) solution of 1H,1'H-[2,4']Biimidazolyl-4-carbaldehyde (1.95 g) and 18-crown-6 (634 mg) at 0°C. The reaction mixture was stirred for 10 min and treated with methyl iodide (1.85 mL). After stirring for 30 at °C and then 17 h at room temperature, The reaction mixture was concentrated under reduced pressure. The residue was dissolved with H_2O-$CHCl_3$ and separated. The aqueous layer was extracted with chloroform. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduced pressure. The crude material was purified with silica gel column chromatography (chloroform - methanol = 9 / 1). The title compound (887 mg, 39%) and its positional isomer 1,1'-Dimethyl-1H,1'H-[2,4']biimidazolyl-4-carbaldehyde (296 mg, 13%) were obtained as a pale pink solid.

3,1'-Dimethyl-3H,1'H-[2,4']biimidazolyl-4-carbaldehyde: ^{1}H NMR (δ, $CDCl_3$) 3.78 (s, 3H), 4.37 (s, 3H), 7.51 (d, 1H, J = 1.0 Hz), 7.62 (d, 1H, J = 1.0 Hz), 7.76 (s, 1H), 9.71 (s, 1H).

1,1'-Dimethyl-1H,1'H-[2,4']biimidazolyl-4-carbaldehyde : ^{1}H NMR (δ, $CDCl_3$) 3.76 (s, 3H), 4.10 (s, 3H), 7.47 (d, 1H, J = 0.9 Hz), 7.57 (s, 1H), 7.59 (d, 1H J = 0.9 Hz), 9.85 (s, 1H).

Step 2: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(3,1'-dimethyl-3H,1'H-[2,4']biimidazolyl-4-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

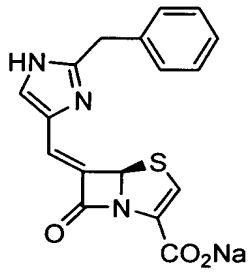
3,1'-Dimethyl-3H,1'H-[2,4']biimidazolyl-4-carbaldehyde (171 mg) was added to the dry acetonitrile (20 mL) solution of anhydrous $MgBr_2$ (800 mg) under a nitrogen atmosphere at room temperature. The dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (346

mg) was added to the mixture, cooled to –20 °C, and triethylamine (0.768 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with 4-dimethylaminopyridine (22 mg) and acetic anhydride (0.17 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 17 h at 0°C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with chloroform - methanol (9/1). The title compound was obtained (507.6 mg, 91.4%). ^{1}H NMR (δ, $CDCl_3$) 2.19 (s, 3H), 3.75 (s, 3H), 4.03 (s, 3H), 5.29 (d, 1H, *J* = 13.5 Hz), 5.42 (d, 1H, *J* = 13.5 Hz), 6.03 (s, 1H), 6.65 (s, 1H), 7.34 (s, 1H), 7.48 (d, 1H, *J* = 1.2 Hz), 7.55 (d, 1H, *J* = 1.2 Hz), 7.59-7.61 (m, 3H), 8.23-8.26 (m, 2H).

Step 3: (5*R*), (6*Z*)-6-(3,1'-Dimethyl-3H,1'H-[2,4']biimidazolyl-4-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(3,1'-dimethyl-3H,1'H-[2,4']biimidazolyl-4-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (596 mg) was dissolved in THF (8.3 mL) and acetonitrile (3.9 mL). Freshly activated Zn dust (2.38 g) and 0.5 *M* phosphate buffer (pH 6.5, 12.2 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (60 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5% - 10% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (66.3 mg, 19%). Mp 113 °C (dec); ^{1}H NMR (δ, D_2O) 3.31 (s, 3H), 3.52 (s, 3H), 5.74 (s, 1H), 6.43 (s, 2H), 6.70 (s, 1H), 7.27 (s, 1H), 7.46 (s 1H)

Example 3



Step 1: 2-Benzyl-1*H*-imidazole-4-carbaldehyde

2-Phenyl-acetoamidine (2 g) was added to the solution of 2-bromo-3-isopropoxy-propenal (2.88 g) in $CHCl_3$ (30 mL). The reaction mixture was stirred for 1 h at room temperature. Then triethylamine (2.09 mL) was added to the mixture and heated to reflux for 6 h. The mixture was cooled to room temperature, diluted with $CHCl_3$, and washed with 10% potassium hydrogen carbonate. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (3/1). The crude compound was crystallized from ethyl acetate and *n*-hexane to give the title compound (1.10 g, 40%). ^1H NMR (δ, $CDCl_3$) 4.17 (s, 2H), 7.24-7.36 (m, 5H), 7.72 (brs, 1H), 9.60 (brs, 1H), 10.28 (brs, 1H).

Step 2: 2-Benzyl-4-formyl-imidazole-1-carboxylic acid 4-nitro-benzyl ester

2-Benzyl-1*H*-imidazole-4-carbaldehyde (1 g) and sodium hydrogen carbonate (1.13 g) were dissolved in dioxane (30 mL) and water (30 mL). The 48.7% solution of *p*-nitrobenzyl chloroformate (PNZCl) in dioxane (2.62 g) was added to the solution at 0 °C and stirred for 2.5 h. The reaction mixture was diluted with ethyl acetate and washed with saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with chloroform - acetone (9/1). The title compound was obtained as pale brown oil (1.97 g, 100%). ^1H NMR (δ, $CDCl_3$) 4.46 (s, 2H), 5.42 (s, 2H), 7.19-7.30 (m, 5H), 7.44-7.47 (m, 2H), 8.06 (s, 1H), 8.21-8.25 (m, 2H), 9.91 (s, 1H).

Step 3: (5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[2-benzyl-1-(4-nitro-benzyloxycarbonyl)-1*H*-

imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

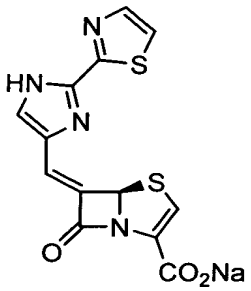
The solution of 2-benzyl-4-formyl-imidazole-1-carboxylic acid 4-nitro-benzyl ester (1.9 g) in dry acetonitrile (10 mL) was added to the dry acetonitrile (50 mL) solution of anhydrous $MgBr_2$ (2.41 g) under a nitrogen atmosphere at room temperature. The dry THF solution (60 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (2 g) was added to the mixture, cooled to –20 °C, and triethylamine (1.8 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with 4-dimethylaminopyridine (127 mg) and acetic anhydride (0.98 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with H_2O, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (2/3 ~ 1/1). The title compound was obtained as two diastereo mixture (3/2, a pale yellow amorphous solid, 2.8 g, 70%). ^{1}H NMR (δ, $CDCl_3$) 2.00 (s, 0.6 x 3H), 2.26 (s, 0.4 x 3H), 4.34-4.37 (m, 2H), 5.25-5.47 (m, 4H), 6.02 (s, 0.4 x 1H), 6.22 (s, 0.6 x 1H), 6.25 (s, 0.6 x 1H), 6.85 (d, 0.4 x 1H, *J* = 1.5 Hz), 7.14-7.62 (m, 11H), 8.21-8.25 (m, 4H).

<u>Step 4:</u> (5*R*), (6*Z*)-6-(2-Benzyl-1*H*-imidazol-4-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[2-benzyl-1-(4-nitro-benzyloxycarbonyl)-1*H*-imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (2.5 g) was dissolved in THF (35 mL) and acetonitrile (16.3 mL). Freshly activated Zn dust (10 g) and 0.5 *M* phosphate buffer (pH 6.4, 51.3 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The mixture was cooled to 9 °C, and 1 *M* NaOH aqueous solution was added to adjust pH to 7.5. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to

Diaion HP-21 (250 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5-10% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (780 mg, 67%). Mp 146 °C (dec); ^{1}H NMR (δ, D_2O) 3.92 (s, 2H), 6.39 (d, 1H, J = 0.8 Hz), 6.74 (s, 1H), 6.89 (s, 1H), 7.13-7.16 (m, 3H), 7.21-7.25 (m, 3H).

Example 4



Step 1: 4-Formyl-2-thiazol-2-yl-imidazol-1-carboxylic acid 4-nitro-benzyl ester

2-Thiazol-2-yl-1*H*-imidazol-4-carbaldehyde (570 mg) was dissolved in dry CH_2Cl_2 (35 mL). *N*,*N*'-Diisopropylethylamine and 48.7% solution of *p*-nitrobenzyl chloroformate (PNZCl) in dioxane (1.69 mL) were added successively to the solution at 0 °C and stirred for 2 h. The reaction mixture was diluted with $CHCl_3$ and washed with saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was crystallized from ethyl acetate and *n*-hexane to give the title compound (1.05 g, 92%). ^{1}H NMR (δ, $CDCl_3$) 5.49 (s, 2H), 7.50 (d, 2H, J = 8.8 Hz), 7.55 (d, 1H, J = 3.4 Hz), 7.89 (d, 1H, J = 3.1 Hz), 8.19 (s, 1H), 8.24 (dq, 2H, J = 8.8, 2.0 Hz), 9.97 (s, 1H).

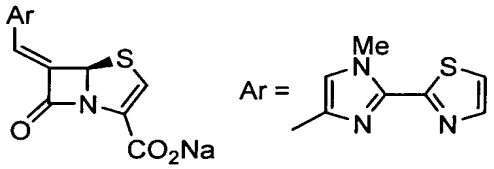
Step 2: (5*R*), (6*Z*)-6-(2-thiazol-2-yl-1*H*-imidazol-4-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

4-Formyl-2-thiazol-2-yl-imidazol-1-carboxylic acid 4-nitro-benzyl ester (940 mg) was added to the dry acetonitrile (35 mL) solution of anhydrous $MgBr_2$ (1.26 g) under a nitrogen atmosphere at room temperature. The dry THF solution (28 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

(1.01 g) was added to the mixture, cooled to –20 °C, and triethylamine (0.942 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2.8 h at –20 °C and treated with 4-dimethylaminopyridine (64 mg) and acetic anhydride (0.495 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 20 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 0.1 M phosphate buffer (pH 7), saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was roughly purified by silica gel column chromatography (ethyl acetate - hexane = 1/1 ~ ethyl acetate only) and the crude (5*R*, 6*RS*)-6-{(*RS*)-acetoxy-[1-(4-nitro-benzyloxycarbonyl)-2-thiazol-2-yl-1*H*-imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (860 mg) was obtained.

Step 3: The crude (5*R*, 6*RS*)-6-{(*RS*)-acetoxy-[1-(4-nitro-benzyloxycarbonyl)-2-thiazol-2-yl-1*H*-imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (860 mg) was dissolved in THF (12 mL) and acetonitrile (5.6 mL). Freshly activated Zn dust (3.44 g) and 0.5 *M* phosphate buffer (pH 6.4, 17.6 mL) were added to the solution. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The mixture was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (90 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 2.5-5% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (126 mg, 11% from **7**). Mp 145 °C (dec); ^{1}H NMR (δ, D_2O) 6.40 (d, 1H, *J* = 0.7 Hz), 6.81 (s, 1H), 6.89 (s, 1H), 7.38 (s, 1H), 7.48 (d, 1H, *J* = 3.1 Hz), 7.71 (d, 1H, *J* = 3.3 Hz).

Example 5



Step 1: 1-Methyl-2-thiazol-2-yl-1*H*-imidazol-4-carbaldehyde

Potassium tert-butoxide (494 mg) was added to the dry THF (30 mL) and dry DMF (10 mL) mixed solution of 2-thiazol-2-yl-1*H*-imidazol-4-carbaldehyde (717 mg) and 18-crown-6 (106 mg) at room temperature. The reaction mixture was stirred for 10 min and treated with methyl iodide (0.274 mL). After stirring for 17.5 h at room temperature, it was concentrated under reduced pressure. The residue was dissolved with ethyl acetate and filtered. The filtrate was evaporated under reduced pressure. The crude material was purified with silica gel column chromatography (ethyl acetate - hexane = 1 / 1). The title compound (410 mg, 53%) and its positional isomer 1-methyl-2-thiazol-2-yl-1*H*-imidazol-5-carbaldehyde (240 mg, 31%) were obtained as a white solid.

1-Methyl-2-thiazol-2-yl-1*H*-imidazol-4-carbaldehyde: ^{1}H NMR (δ, $CDCl_3$) 4.21 (s, 3H), 7.44 (d, 1H, J = 3.2 Hz), 7.68 (s, 1H), 7.89 (d, 1H, J = 3.2 Hz), 9.91 (s, 1H).

1-methyl-2-thiazol-2-yl-1*H*-imidazol-5-carbaldehyde: ^{1}H NMR (δ, $CDCl_3$) 4.48 (s, 3H), 7.51 (d, 1H, J = 3.1 Hz), 7.82 (s, 1H), 7.97 (d, 1H, J = 3.1 Hz), 9.82 (s, 1H).

Step 2: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(1-methyl-2-thiazol-2-yl-1*H*-imidazol-4-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

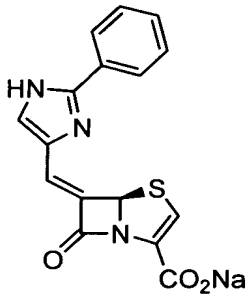
1-Methyl-2-thiazol-2-yl-1*H*-imidazol-4-carbaldehyde (380 mg) was added to the dry acetonitrile (28 mL) solution of anhydrous $MgBr_2$ (1.03 g) under a nitrogen atmosphere at room temperature. Colorless powder was deposited over 10 min. The dry THF solution (28 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (760 mg) was added to the mixture, cooled to –20 °C, and triethylamine (0.768 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with 4-dimethylaminopyridine (48 mg) and acetic anhydride (0.371 mL) in

one portion. The reaction mixture was warmed to 0 °C and stirred for 22 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (3/2). The title compound was obtained as two diastereo mixture (1/1, a pale yellow amorphous solid, 673.4 mg, 52%). ^{1}H NMR (δ, $CDCl_3$) 2.00 (s, 0.5 x 3H), 2.28 (s, 0.5 x 3H), 4.12 (s, 3H), 5.28 (d, 0.5 x 1H, *J* = 13.5 Hz), 5.29 (d, 0.5 x 1H, *J* = 13.5 Hz), 5.44 (d, 0.5 x 1H, *J* = 13.5 Hz), 5.47 (d, 0.5 x 1H, *J* = 13.5 Hz), 6.16 (s, 0.5 x 1H), 6.30 (s, 0.5 x 1H), 6.42 (s, 0.5 x 1H), 6.87 (d, 0.5 x 1H, *J* = 0.8 Hz), 6.92 (d, 0.5 x 1H, *J* = 0.8 Hz), 7.19 (s, 0.5 x 1H), 7.34-7.36 (m, 1H), 7.47 (s, 0.5 x 1H), 7.50 (s, 0.5 x 1H), 7.60-7.64 (m, 2H), 7.83-7.85 (m, 1H), 8.23-8.26 (m, 1H).

Step 3: (5*R*), (6*Z*)-6-(1-Methyl-2-thiazol-2-yl-1H-imidazol-4-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(1-methyl-2-thiazol-2-yl-1*H*-imidazol-4-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (604 mg) was dissolved in THF (8.5 mL) and acetonitrile (3.9 mL). Freshly activated Zn dust (2.41 g) and 0.5 *M* phosphate buffer (pH 6.4, 23.6 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (60 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5% acetonitrile-water and 10% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (231 mg, 64%). Mp 214 °C (dec); ^{1}H NMR (δ, D_2O) 3.91 (s, 3H), 6.44 (s, 1H), 6.84 (s, 1H), 6.91 (s, 1H), 7.43 (s, 1H), 7.60 (d, 1H, *J* = 3.3 Hz), 7.84 (d, 1H, *J* = 3.3 Hz).

Exampl 6



Step 1: 4-Formyl-2-phenyl-imidazole-1-carboxylic acid 4-nitro-benzyl ester

4-Formyl-2-phenylimidazole (624 mg) and sodium hydrogen carbonate (791 mg) were dissolved in dioxane (3.6 mL), THF (3.6 mL) and water (7.2 mL). The 48.7% solution of *p*-nitrobenzyl chloroformate (PNZCl) in dioxane (2.08 mL) was added to the mixture at room temperature and stirred for 2.5 h. The mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was crystallized from ethyl acetate and *n*-hexane to give the title compound (956 mg, 75%). ^1H NMR (δ, $CDCl_3$) 5.41 (s, 2H), 7.32 (d, 2H, J = 8.6 Hz), 7.40-7.51 (m, 3H), 7.56-7.58 (m, 2H), 8.17-8.20 (m, 2H), 8.22 (s, 1H), 9.97 (s, 1H).

Step 2: (5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[1-(4-nitro-benzyloxycarbonyl)-2-phenyl-1*H*-imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

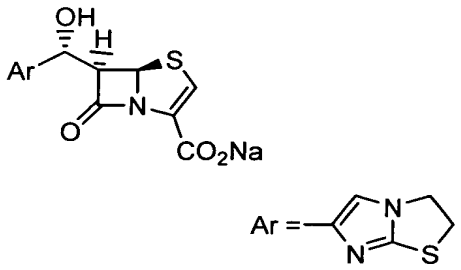
4-Formyl-2-phenyl-imidazole-1-carboxylic acid 4-nitro-benzyl ester (568 mg) and the dry THF solution (15 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (587 mg) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$ (622.4 mg) under a nitrogen atmosphere at room temperature. After cooling the mixture to –20 °C, triethylamine (0.494 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 1.5 h at –20 °C and

treated with acetic anhydride (0.277 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 27 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (2/3 ~ 1/1). The title compound was obtained as three diastereo mixture (6/4/1.5, a pale yellow amorphous solid, 986 mg, 86%). ^{1}H NMR (δ, $CDCl_3$) 2.04 (s, 0.35 x 3H), 2.10 (s, 0.13 x 3H), 2.30 (s, 0.52 x 3H), 5.25-5.43 (m, 4H), 6.09 (s, 0.52 x 1H), 6.22 (s, 0.13 x 1H), 6.31 (s, 0.35 x 1H), 6.33 (s, 0.35 x 1H), 6.87 (s, 0.13 x 1H), 6.92 (d, 0.52 x 1H, J = 1.4 Hz), 7.31-7.76 (m, 11H), 8.17-8.25 (m, 4H).

Step 3: (5*R*), (6*Z*)-6-(2-phenyl-1*H*-imidazol-4-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

(5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[1-(4-nitro-benzyloxycarbonyl)-2-phenyl-1*H*-imidazol-4-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.15 g) was dissolved in THF (16.1 mL) and acetonitrile (7.5 mL). Freshly activated Zn dust (4.6 g) and 0.5 *M* phosphate buffer (pH 6.4, 23.6 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (100 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5-10% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (322 mg, 63%). Mp 281 °C (dec); ^{1}H NMR (δ, D_2O) 6.32 (s, 1H), 6.76 (s, 1H), 6.79 (s, 1H), 7.22 (s, 1H), 7.24-7.33 (m, 3H), 7.60-7.63 (m, 2H).

Example 7



Step 1: (5*R*, 6*S*)-6-Bromo-6-[(*S*)-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-hydroxy-methyl]-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

2,3-Dihydro-imidazo[2,1-b]thiazole-6-carbaldehyde (2.63 g) was added to the dry acetonitrile (124 mL) solution of anhydrous $MgBr_2$ (3.8 g) under a nitrogen atmosphere at room temperature. Colorless powder was deposited over 30 min. The dry THF solution (124 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl este (6.2 g) was added to the mixture. After cooling the mixture to –20 °C, triethylamine (5.21 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2.5 h at –20 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (3/1 ~ ethyl acetate only). The mixture of the title compound **3** and its diastereo isomer was obtained (10/1, a pale brown amorphous solid, 6.63 g, 79%) and the title compound (3.20 g, 38%) was separated from the mixture by silica gel column chromatography. ^1H NMR (δ, $CDCl_3$) 3.80-3.89 (m, 2H), 4.15-4.25 (m, 2H), 5.22 (s, 1H), 5.31 (d, 1H, J = 13.5 Hz), 5.45 (d, 1H, J = 13.5 Hz), 6.12 (s, 1H), 7.12 (s, 1H), 7.42 (s, 1H), 7.62 (d, 2H, J = 8.6 Hz), 8.22-8.25 (m, 2H).

Step 2: (5*R*, 6*S*)-6-[(*S*)-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-yl)-hydroxy-methyl]-7-

oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

(5*R*, 6*S*)-6-Bromo-6-[(*S*)-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-hydroxy-methyl]-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (2.0 g) was dissolved in THF (28 mL) and acetonitrile (13 mL). Freshly activated Zn dust (8.0 g) and 0.5 *M* phosphate buffer (pH 6.4, 41 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The mixture was cooled to 3 °C, and 1 *M* NaOH aqueous solution was added to adjust pH to 7.5. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (200 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 2.5% acetonitrile-water. The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a pale yellow amorphous solid (171 mg, 13%). Mp 152 °C; ^{1}H NMR (δ, D_2O) 3.91 (t, 2H, J = 7.4 Hz), 4.23 (t, 2H, J = 7.4 Hz), 4.33-4.35 (m, 1H), 5.07 (d, 1H, J = 5.5 Hz), 5.70 (d, 1H, J = 1.6 Hz), 7.05 (d, 1H, J = 1.0 Hz), 7.24 (s, 1H).

Example 8

Preparation of (5R,6Z)-6-[(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step1: Ethyl 5-benzoyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylate:

To a stirred dry DMF (7.3 g, 100 mmol), $POCl_3$ (12.25 g, 80 mmol) was slowly added between 0°C to 5° C. After the addition the solidified mass was dissolved in CH_2Cl_2 (20 ml) and stirred at room temperature for 2 hrs. Again the temperature was cooled to 0°C and 1-benzoyl-4-piperidone in CH_2Cl_2 was added slowly. After the addition the reaction mixture was stirred at room temperature for 2 hrs and poured over crushed ice and sodium acetate. It was stirred for 30 minutes at room temperature. Extracted with CH_2Cl_2; washed well with water; dired over anhydrous $MgSO_4$ and concentrated. The crude product was dissolved in CH_2Cl_2 and ethylmercaptoacetae (9.6 g, 80 mmol) / Et_3N (10.1 g, 100 mmol) was added slowly at room temperature. The reaction mixture was

refluxed for 2 hrs and quenched with water. CH_2Cl_2 layer was washed well with water; dried over anhydrous $MgSO_4$; filtered and concentrated. The product was purified by SiO_2 column chromatography by elting it with 50% ethylacetae; hexane. Yellow oil; Yield: 6.4 gms (25%); M+H 316.

Step 2: (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methanol:

To stirred suspension of LAH (2.0 gms) a solution of ethyl 5-benzoyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylate (6.0 g, 19 mmol) in THF was added slowly at 0^0 C. After addition reaction mixture was stirred for 30 minutes and quenched with saturated NH_4Cl. It was diluted with $CHCl_3$ and filtered. The fitrate was washed with saturated brine solution and dried over anhydrous $MgSO_4$. It was filtered and taken to next step with out purifications. Yield: 4.5 g 91%. Yellow liquid.

Step 3: 2-Formyl (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine:

To a stirred solution of (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methanol (4.0 g, 15.4 mmol) in CH_2Cl_2 (300 ml) active MnO_2 (20 g, excess) was added and stirred at room temperature for 18 hrs. At the end, the reaction mixture was filtered through celite and washed with $CHCl_3$. Reaction mixture was washed well with water; dried and concentrated. The product was found to be pure and taken to next step with oiut purifications. Yield: 3.0 g (76%); (M+H: 257).

Step 4: 4-Nitrobenzy-6-[(acetyloxy)(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl (5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (565 mg, 2.2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5%

citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 550 mg, 40%; M+H 687.

Step-5: (5R,6Z)-6-[(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)(5-benzyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate(450 mg, 0.65 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 18%; as yellow crystals; mp. 198^{0}C; (M+H) 411 .

^{1}H NMR (DMSO-d_6) d 2.7 (m, 2H), 2.8 (bm, 2H), 3.4 (m, 2H), 3.8 (s, 2H), 6.3 (s, 1H), 6.5 (s, 1H), 7.1(s, 1H), 7.28(s, 1H), 7.4 (s, 5H).

Example 9

Preparation of (5*R*),(6*Z*)-6-(7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Imidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester:

Ethyl bromopyruvate (62.9 g) was added to the DME (258 mL) solution of 2-aminopyrazine (24.8 g) at room temperature and stirred for 2.5 h. The reaction mixture was cooled to 0 °C and stirred for 30 min to afford a pale brown precipitate. The precipitate was filtered and washed with Et_2O to give pale brown crystals. The suspension of the precipitate (66.1 g) in EtOH (1.29 L) was heated at reflux temperature to turn to clear solution. After refluxing for 2h, the reaction mixture was concentrated under reduced pressure, then mixed with $CHCl_3$ and saturated $NaHCO_3$aq. The mixture was filtered through a pad of Celite and the separated organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (99/1 ~ 97/3), and collected fractions were concentrated under reduced pressure followed by recrystallization from $CHCl_3$ - Et_2O. The titled compound was obtained as pale pink crystals. Yield: 10.9 g, 22%).

^{1}H NMR($CDCl_3$) d 1.46(t, 3H, *J* = 7.2 Hz), 4.49(q, 2H, *J* = 7.2 Hz), 7.96(d, 1H, *J* = 4.7 Hz), 8.08(dd, 1H, *J* = 1.2, 4.7 Hz), 8.26(s, 1H), 9.21(d, 1H, *J* = 1.2 Hz).

Step 2: 5,6,7,8-Tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester, Hydrochloride:

0.46 *M* HCl - EtOH (169 mL) and 10% Pd-C (50% wet) (1.37 g) were added to the EtOH (546 mL) solution of imidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (13.7 g). The mixture was hydrogenated under H_2 at 40 psi at room temperature for 15 h. The reaction mixture was filtered and Pd-C was washed with EtOH. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (9/1 ~ 2/1). The titled compound was obtained as brown crystals Yield: 10.4 g, 63%.

^{1}H NMR($CDCl_3$) d 1.38(t, 3H, *J* = 7.1 Hz), 3.90(t, 2H, *J* = 5.7 Hz), 4.40(q, 2H, *J* = 7.1 Hz), 4.59(t, 2H, *J* = 5.7 Hz), 4.80(s, 2H), 8.20(s, 1H).

Step 3: 7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester:

Et_3N (3.44 mL), 37% HCHO aq. (2.02 mL) and $NaBH_3CN$ (1.78 g) were added successively to the MeOH (75 mL) solution of 5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-

2-carboxylic acid ethyl ester, hydrochloride (5.2 g) at room temperature and stirred for 3.5 h under a nitrogen atmosphere. The mixture was diluted with CH_2Cl_2 and washed with 50% K_2CO_3 aq. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (1/1 ~ 1/2). The titled compound was obtained as orange oil. Yield: 2.68 g, 57%).

^{1}H NMR($CDCl_3$) d 1.37(t, 3H, *J* = 7.1 Hz), 2.50(s, 3H), 2.85(t, 2H, *J* = 5.5 Hz), 3.69(s, 2H), 4.06(t, 2H, *J* = 5.5 Hz), 4.36(t, 2H, *J* = 7.1 Hz), 7.52(s, 1H).

Step 4: 7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde:

1.01 *M* solution of DIBAL in toluene (13.6 mL) was added to the dry CH_2Cl_2 (86 mL) solution of 7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (1.8 g) under a nitrogen atmosphere at –78 °C and stirred for 2 h. The mixture was quenched with 1*M* HCl. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with 50% K_2CO_3 aq. and the aqueous layer was extracted with CH_2Cl_2 three times. The combined organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - MeOH (19/1 ~ 9/1). The titled compound **5** was obtained as colorless crystals. Yield: 591 mg, 42%).

^{1}H NMR($CDCl_3$) d 2.51(s, 3H), 2.87(t, 2H, *J* = 5.5 Hz), 3.70(s, 2H), 4.10(t, 2H, *J* = 5.5 Hz), 7.53(s, 1H), 9.82(d, 1H, *J* = 1.4 Hz).

Step 5: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (diastereo mixture):

7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde (1.19 g) was added to the dry acetonitrile (97 mL) solution of anhydrous $MgBr_2$ (4.05 g) under a nitrogen atmosphere at room temperature. The dry THF solution (97 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (3.32 g) was added to the mixture, cooled to –20 °C, and Et_3N (3.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4.5 h at –20 °C and treated with acetic anhydride (1.36 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 17 h at 0 °C. The

mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (9/1 ~ 2/1). The titled compound was obtained as two diastereo mixture. Red oil, Yield: 1.13 g.

^1H NMR($CDCl_3$) d 1.20(s, 0.81x3H), 2.24(s, 0.19x3H), 2.48(s, 3H), 2.80 ~ 2.84(m, 2H), 3.57 ~ 3.67(m, 2H), 3.97 ~ 4.02(m, 2H), 5.27(d, 1H, *J* = 13.6 Hz), 5.42(d, 0.19x1H, *J* = 13.6 Hz), 5.45(d, 0.81x1H, *J* = 13.6 Hz), 6.07(s, 0.19x1H), 6.30(s. 0.81x2H), 6.79(s, 0.19x1H), 6.80(s, 0.19x1H), 7.02(s, 0.81x1H), 7.44(s, 0.19x1H), 7.47(s, 0.81x1H), 7.60(d, 0.19x2H, *J* = 8.2 Hz), 7.62(d, 0.81x2H, *J* = 8.6 Hz), 8.22 ~ 8.26(m, 2H).

Step 6: (5*R*),(6*Z*)-6-(7-Methyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt.

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-methyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (1.11 g) was dissolved in THF (32 mL) and acetonitrile (32 mL). Freshly activated Zn dust (4.46 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 48 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered through a pad of Celite, cooled to 3 °C, and 1 *M* NaOH was added to adjust pH to 7.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (20 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 95/5). The combined fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid. Yield: 417 mg, 65%: mp 200 °C (dec); ^1H NMR(D_2O) d 2.32(s, 3H), 2.79 ~ 2.81(m, 2H), 3.54(s, 2H), 3.95(t, 2H, *J* = 5.6 Hz), 6.39(s, 1H), 6.85(s, 1H), 6.87(s, 1H), 7.26(s, 1H).

Example 10

Preparation of (5*R*), (6*Z*)-7-Oxo-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

2-Ketopiperazine

2-Ketopiperazine was prepared in the method of Merck group (USP 5,629,322).

Step 1: 4-*p*-Nitrobenzyloxycarbonyl-2-ketopiperazine

The 48.7% solution of *p*-nitrobenzyloxycarbonyl chloride in 1,4-Dioxane (10.7 mL) was added to the dichloromethane (110 mL) solution of 2-Ketopiperazine (2.21 g) and diisopropylethylamine (4.6 mL) at 0°C and stirred for 0.5 h at 0 °C. Water (300 mL) was added to the reaction mixture, and extracted with dichloromethane (3 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (30 : 1), and the title compound was obtained as white solid (7.1 g, quant.).

^1H NMR (d, $CDCl_3$) 3.42-3.45 (m, 2H), 3.74 (t, 2H, J = 5.4 Hz), 4.19 (s, 2H), 5.26 (s, 2H), 6.39 (brs, 1H), 7.52 (d, 2H, J = 8.6 Hz), 8.24 (d, 2H, J = 8.6 Hz).

Step 2: 5-Methoxy-4-*p*-nitrobenzyloxycarbonyl-1,2,3,6-tetrahydropyrazine:

Trimethyloxonium tetrafluoroborate (97%, 3.7 g) was added to the dry dichloromethane (120 mL) solution of 4-*p*-nitrobenzyloxycarbonyl-2-ketopiperazine (6.7 g) at room temperature and stirred for 17 h. The reaction mixture was treated with saturated sodium hydrogen carbonate aqueous solution, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 100 mL), then the combined organic layer was washed with saturated sodium hydrogen carbonate aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure and the title compound was obtained as a pale brown solid. Yield; 5.7 g, 80.6.

^1H NMR (d, $CDCl_3$) 3.48 (m, 2H), 3.57 (m, 2H), 3.70 (s, 3H), 3.97 (s, 2H), 5.26 (s, 2H), 7.52 (d, 2H, J = 8.7 Hz), 8.23 (d, 2H, J = 8.7 Hz).

Step 3: 2-Imino-4-*p*-nitrobenzyloxycarbonyl piperazine:

The mixture of 5-methoxy-4-*p*-nitrobenzyloxycarbonyl-1,2,3,6-tetrahydropyrazine (5.7 g) and ammonium chloride (1.6 g) in dry ethanol (100 mL) was heated to reflux for 4 h. The reaction mixture was then concentrated under reduce pressure. Dichloromethane (100 mL) was added to the residue and extracted with water (3 x 50 mL) then the combined aqueous layer was washed with dichloromethane. The aqueous layer was neutralized with 10% potassium carbonate aqueous solution and then extracted with dichloromethane (8 x 50 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure and the title compound was obtained as a white solid. Yield: 4.9 g, 91.2%.

^{1}H NMR (d, $CDCl_3$) 3.49 (brs, 4H), 3.98 (brs, 2H), 5.26 (s, 2H), 7,52 (d, 2H, J = 8.6 Hz), 8.23 (d, 2H, J = 8.6 Hz).

Step 4: 7-*p*-Nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine-2-carbaldehyde (9) and 7-*p*-nitrobenzyloxycarbonyl-5,6,7,8 tetrahydroimidazo[1,2-a]pyrazine-3-carbaldehyde:

The mixture of 2-bromo-3-hydroxypropenal (2.8 g), p-toluenesulfonic acid monohydrate (33 mg) and 2-propanol (3.5 mL) in cyclohexane (28 mL) was azeotroped until the vaper temperature rose to 80°C. The reaction mixture was concentrated under reduce pressure. The residue was dissolved in dry acetonitrile (30 mL). The dry acetonitrile (310 mL) solution of 2-imino-4-*p*-nitrobenzyloxycarbonyl piperazine (4.7 g) was added at room temperature. The reaction mixture was stirred at room temperature for 3 h, and then the reaction solution was removed in vacuo. The residue was dissolved in ethyl acetate (170 mL) and triethylamin (2.4 mL) was added, then the reaction mixture was heated to reflux for 1.5 h. The reaction mixture was cooled to room temperature, and then water (170 mL) was added to the reaction mixture and separated. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (50 : 1), and the title compound was obtained as a brown solid, (Yield: 2.9 g, 51.6%) and its regio isomer (orange amorphous solid, Yield; 0.8 g, 14.9%) were obtained.

^{1}H NMR (d, $CDCl_3$) 3.99 (t, 2H, J = 5.4 Hz), 4.14 (t, 2H, J = 5.4 Hz), 4.85 (s, 2H), 5.29 (s, 2H), 7.54 (d, 2H, J = 8.6 Hz), 7.57 (s, 1H), 8.24 (d, 2H, J = 8.6 Hz), 9.85 (s, 1H).

Regio isomer ^{1}H NMR (d, $CDCl_3$) 3.95 (t, 2H, *J* = 5.4 Hz), 4.44 (t, 2H, *J* = 5.4 Hz), 4.87 (s, 2H), 5.29 (s, 2H), 7.54 (d, 2H, *J* = 8.7 Hz), 7.78 (s, 1H), 8.24 (d, 2H, *J* = 8.7 Hz), 9.71 (s, 1H).

Step 5: (5R)-6-[Acetoxy-(7-p-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid p-nitrobenzyl ester:

The dry acetonitrile (25 mL) solution of 7-*p*-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine-2-carbaldehyde (1.6 g) was added to the dry acetonitrile (55 mL) solution of $MgBr_2$ (2.2 g) under an nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (80 mL) solution of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.8 g) was added, the mixture was cooled to –20 °C then triethylamine (1.6 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4,4-dimethylamino pyridine (58.3 mg) and acetic anhydride (0.89 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. 10% Citric acid aqueous solution (320 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 160 mL). The organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with CH_2Cl_2 – acetone (20 : 1), and the title compound was obtained as two diastereo mixture (81 : 19, brown foamy amorphous solid. Yield: 2.1 g, 59.9%.

^{1}H NMR (d, $CDCl_3$) 2.01 (s, 2.43H), 2.24 (s, 0.57H), 3.93-,3.96 (m, 2H), 4.02-4.05 (m, 2H), 4.74-4.76 (m, 2H), 5.28 (d, 1H, *J* = 13.5 Hz), 5.28 (s, 2H), 5.45 (d, 1H, *J* = 13.5 Hz), 6.07 (s, 0.19H), 6.29 (s, 0.81H), 6.31 (s, 0.81H), 6.80 (s, 0.19H), 6.83 (s, 0.19H), 7.08 (s, 0.81H), 7.43 (s, 0.19H), 7.46 (s, 0.81H), 7.54 (d, 2H, *J* = 8.6 Hz), 7.61 (d, 2H, *J* = 8.8 Hz), 8.24 (d, 4H, *J* = 8.3 Hz).

Step 6: (5*R*), (6*Z*)-7-Oxo-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

(5R)-6-[Acetoxy-(7-p-nitrobenzyloxycarbonyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid p-

nitrobenzyl ester (2.0 g) was dissolved in THF (63 mL). Freshly activated Zn dust (7.9 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 63 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was filterd through a pad of Celite and the pad was washed with water (150 mL) and *n*-butanol (150 mL). The aqueous layer was separated and then the organic layer was extracted with water (2 x 50 mL). The combined aqueous layer was concentrated to 61 g and applied to Diaion HP-21 resin (80 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid. Yield: 172 mg, 20.1%: mp 150 °C (dec); ^{1}H NMR (d, D_2O) 3.02 (t, 2H, J = 5.6 Hz), 3.82 (s, 2H), 3.89 (d, 2H, J = 5.6 Hz), 6.38 (s, 1H), 6.84 (s, 1H), 6.87 (s, 1H), 7.24 (s, 1H); IR (KBr)

Example 11

Preparation of (5R,6Z)-6-{[5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: 5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate: 5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate was prepared according to the procedure as outlined in Example 5, (Step 1). Starting from tert-butyl-1-piperidinecarboxylate (9.9 g, 50 mmol), $POCl_3$ (6.3 g, 40 mmol) and DMF (3.8 g, 50 mmol). The chloro formyl intermediate was reacted with ethyl mercaptoacetate (6.0 g, 50 mmol) and Et_3N. The product was purified by SiO_2 column chromatography by eluting it with 3:1 hexane; ethylacetae. Yield: 8.7 g, 56%; White liquid. (M+H) 312.

Step 2: tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate:

tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate was prepared according to the procedure outlined in Example 5, (Step 2). Starting from 5-tert-butyl 2-ethyl 6,7-dihydrothieno[3,2-c]pyridine-2,5(4H)-dicarboxylate (1.0 g, 3.21 mmol) and $LiAlH_4$ (500 mg , excess), 807 mg (92% yield) of the alcohol derivative was

isolated as white liquid. (M+H) 270.

Step 3: tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate:
tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate was prepared according to the procedure outlined in Example 5, (Step 3). Starting from tert-butyl 2-(hydroxymethyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate (1.0 g 3.7 mmol) in methylene chloride (100 ml) and active MnO_2 (5 g, excess), 800 g (81% Yield) of the aldehyde derivative was isolated as brown solid. (M+H) 268.

Step 4: 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine:
2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine was prepared starting from tert-butyl 2-(formyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate (1.0 g 3.7 mmol) was dissolved in CH_2Cl_2 (20 ml), MeOH (90% 20 ml) and 1N. HCl in dioxane (10 ml). The reaction mixture was stirred at room temperature for 48 hrs. At the end reaction mixture was concentrated to dryness and taken to next step with out purification. Yield: 750 mg (HCl salt, Quantitative); M+H 168.

Step 5: 2-Formyl [5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (1.4 g, 5.2 mmol) in DMF (20 ml) , 4-methoxybenzyl chloride (0.94 g, 6.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow oil. Yield: 470 mg , 35%; M+H 288.

Step 6: 4-Nitrobenzy-6-[(acetyloxy)[5(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:
2-Formyl [5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (574 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-

bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 550 mg, 40%; M+H 714 and 716.

Step-7: (5R,6Z)-6-{[5-(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(4-methoxybenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (300 mg, 0.42 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 18%; as yellow crystals; mp. 127^{0}C; (M+H) 441 .

^{1}H NMR (DMSO-d_6) d 2.7 (m, 2H), 2.8 (bm, 2H), 3.4 (m, 2H), 3.74 (s, 3H) 3.8 (s, 2H), 6.6 (s, 1H), 6.88 (dd, 2H), 7.14(s, 1H), 7.24(dd, 2H), 7.4 (s, 1H), 7.59 (s, 1H).

Example 12

Preparation of (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide

5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide was prepared by the method as outlined in USP 5,629,322.

Step 2: 3-Iminothiomorpholin hydrochloride

5-Methylthio-3,6-dihydro-2*H*-[1,4]thiazine hydroiodide (7.1 g) was dissolved with 10% K_2CO_3 aqueous solution (150 mL) and the aqueous layer was extracted with CH_2Cl_2 (5 x 70 mL). The combined organic layer was dried ($MgSO_4$), filtered and concentrated under reduce pressure. Ammonium chloride (1.7 g) was added to the obtained residue in dry ethanol (128 mL) and heated to reflux for 1 h. The reaction mixture was cooled to room temperature. The reaction solution was removed in vacuo and the Iminothiomorpholin hydrochloride was obtained as brown solid (4.3 g, quant.).

^{1}H NMR (d, DMSO-d^6) 3.15 (t, 2H, J = 5.9 Hz), 3.74 (t, 2H, J = 5.9 Hz), 3.83 (s, 2H), 8.97 (brs, 1H), 9.38 (brs, 1H), 9.99 (brs, 1H).

Step 3: 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde

The mixture of 2-bromo-3-hydroxypropenal (**7**, 4.3 g), *p*-toluenesulfonic acid monohydrate (52 mg) and 2-propanol (5.3 mL) in cyclohexane (43 mL) was azeotroped until the vaper temperature rose to 80°C. The reaction mixture was concentrated under reduce pressure. The residue was dissolved in dry ethanol (28 mL). The mixture of the dry ethanol (143 mL) solution of 3-iminothiomorpholin hydrochloride (4.3 g) and 28% methanol solution of sodium methylate (5.0 mL) were added at room temperature. The reaction mixture was stirred at room temperature for 1 h, and then the reaction solution was removed in vacuo. The residue was dissolved in chloroform (128 mL) and triethylamine (3.6 mL) was added, then the reaction mixture was heated to reflux for 2.5

h. The reaction mixture was cooled to room temperature and then concentrated under reduce pressure. The residue was dissolved with dichloromethane (300 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – acetone (10 : 1), and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde (brown solid, 445 mg, 10.3%) and 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde (brown solid, 872 mg, 20.2%) were obtained.

5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbardehyde: ^{1}H NMR (d, $CDCl_3$) 3.07 (t, 2H, J = 5.7 Hz), 3.95 (s, 2H), 4.33 (t, 2H, J = 5.7 Hz), 7.55 (s, 1H), 9.83 (s, 1H).

5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde: ^{1}H NMR (d, $CDCl_3$) 3.05 (t, 2H, J = 5.7 Hz), 3.98 (s, 2H), 4.61 (t, 2H, J = 5.7 Hz), 7.73 (s, 1H), 9.69 (s, 1H).

Step 4: (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

The dry acetonitrile (20 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-2-carbaldehyde (392 mg) was added to the dry acetinitrile (20 mL) solution of $MgBr_2$ (1.1 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (40 mL) solution of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.0 g) was added and the mixture was cooled to –20 °C then triethylamine (0.8 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3.5 h at –20 °C and treated with 4-dimethylamino pyridine (30 mg) and acetic anhydride (0.44 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14 h at 0 °C. 10% Citric acid aqueous solution (240 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The combined organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was roughly purified by silica gel column chromatography, eluted with CH_2Cl_2 – acetone (50 : 1), and crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as solid.

The solid obtained above was purified by SiO_2 column chromatography

by eluting it with 505 ethylacetae:hexane. The pale yellow solid obtained was dissolved in THF (17 mL). Freshly activated Zn dust (2.2 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 17 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was filterd through a pad of Celite and the pad was washed with water (40 mL) and *n*-butanol (30 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 10 mL). The combined aqueous layer was concentrated to 23 g, 1mol/L NaOH was added to adjust pH to 7.25 and applied to Diaion HP-21 resin (30 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 10% acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give (5*R*), (6*Z*)-6-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt as a yellow amorphous solid (168 mg, 20.9%).

mp 135 °C (dec); ^{1}H NMR (d, D_2O) 3.00 (t, 2H, J = 5.7 Hz), 3.80 (AB, 2H, J = 16.7, 18.1 Hz), 4.19 (t, 2H, J = 5.7 Hz), 6.44 (d, 1H, J = 0.8 Hz), 6.89 (s, 1H), 6.93 (s, 1H), 7.29 (s, 1H); M+H = 322.

Example 13

Preparation of (5*R*), (6*Z*)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde

28% Sodium methoxide (5.26g) was added to the EtOH (250 mL) solution of 4,5-dihydro-3H-pyrrol-2-ylamine hydrochloride (3.27g) at room temperature. After stirring for 5 min at room temperature, 2-bromo-3-propoxy-propenal (5.79g) was added to the mixture at room temperature, then the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was taken to dryness in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and triethylamine (3.8 mL) was added. The mixture was heated to reflux for 3 hours. The reaction mixture was cooled to room temperature, washed with 50% K2CO3, dried over anhydrous K2CO3, filtered, and evaporated under reduced pressure. The residue was applied with silicagel column chromatography,

eluted with CHCl3-acetone (2:1), and 6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde (41%, 1.51g) was obtained as a pale yellow solid.
^{1}H NMR (d, $CDCl_3$): 2.62-2.7 (m, 2H), 2.90-2.94 (m, 2H), 4.07 (t, 2H, J = 7.2 Hz), 7.59 (s, 1H), 9.80 (s, 1H).

Step 2: (5*R*), (6*Z*)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

6,7-Dihydro-5H-pyrrolo[1,2-a]imidazole-2-carbaldehyde (1.36 g) was added to the dry acetonitrile (155 mL) solution of anhydrous $MgBr_2$ (5.64 g) under an argon atmosphere at room temperature. The dry THF solution (155 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (3.86 g) was added to the mixture, cooled to –20 °C, and Et_3N (4.18 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (1.89 mL) and DMAP (370 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14.5 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 1 *M* citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was dissolved in THF (166 mL) and acetonitrile (77 mL). Freshly activated Zn dust (23.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 243 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 *M* NaOH was added to adjust pH to 8. The filtrate was washed with ethyl acetate and the aqueous layer was separated. 1 *M* NaOH was added to the aqueous layer again to adjust pH to 8. The resultant mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (20 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 9/1) to give the purified active fractions of (5*R*), (6*Z*)-6-(6,7-Dihydro-5*H*-pyrrolo[1,2-a]imidazol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt . The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled as a yellow amorphous solid (681 mg, 24%, pH 7.8).
mp 190 °C (dec); ^{1}H NMR(d, D_2O): 2.48-2.56 (m, 2H), 2.74-2.79 (m, 2H), 3.94-3.99 (m,

2H), 6.47 (d, 1H, J = 0.7 Hz), 6.94 (s, 1H), 6.95 (s, 1H), 7.36 (s, 1H); (M+H) 291.

Example 14

Preparation of (5R,6Z)-6-(Imidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Step 1: Ethyl imidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl bromopyruvate (9.8 g, 50 mmol) was added dropwise to a stirred solution of 2-aminobenzothiazole (7.5 g, 50 mmol) in DMF (100 ml) at room temperature. After the addition, the reaction mixture was heated to reflux for 6 h. The reaction mixture was cooled to room temperature and quenched with ice cold water. The aqueous layer was neutralized with NH_4OH and the separated solid was fitered. It was washed well with water and dried. The crude product obtained was taken to next step without purification. Brown solid; Yield: 10 g, 81%; M+H 248. mp 97° C

Step 2: Imidazo[2,1-b]-benzthiazole-2-methanol:

To a stirred slurry of $LiAlH_4$ (2.0 g, excess) in dry THF, ethyl imidazo[2,1-b]-benzthiazole-2-carboxylate (4.9 g, 20 mmol) was slowly added in THF (100 ml) at 0° C. After the addition, the reaction mixture was stirred at room temperature for 1 h and quenched with saturated NH_4Cl/ NH_4OH. The separated solid was diluted with Chloroform/ MeOH (3:1) and filtered through a pad of celite. The organic layer was washed once with saturated NaCl and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The brown solid obtained was taken to next step with out purification. Yield: 3.8 g, 93%; M+H 205; mp 131°C.

Step 3: 2-Formyl-Imidazo[2,1-b]-benzthiazole:

To a stirred solution of imidazo[2,1-b]-benzthiazole-2-methanol (2.04 g, 10 mmol) in methylene chloride (200 ml), activated MnO_2 (15 g, excess) was added. The reaction mixture was stirred at room temperature for 24 h and filtered through a pad of celite. The reaction mixture was concentrated and the product was purified by silica gel column

chromatography by eluting it with 75% ethyl acetate; hexane. Brown solid; Yield: 800 mg, 40%; M+H 203.

Step 4: 4-Nitrobenzyl-6-[(acetyloxy) (imidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-**bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:**

2-Formyl-Imidazo[2,1-b]-benzthiazole (444 mg, 2.2 mmol) and a dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2 mmol) were added successively to a dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$:etherate (619 mg 2.4 mmol) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to the next step. Pale yellow amorphous solid; Yield: 850 mg, 67%; mp 69°C; M+H 630

Step-5: (5*R*),(6*Z*)-6-(Imidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene) -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy) (imidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (500 mg, 0.79 mmol) was dissolved in THF (17 mL) and acetonitrile (36 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 N HCl was added to adjust the pH to 7.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The precipitate was dissolved in acetonitrile and

loaded on a HP-21 reverse phase column. It was eluted with deionized water (2 L) and latter eluted with 10% acetonitrile:water. Yield: 105 mg, 35%; as yellow crystals; mp 233°C; M+H 356.

^{1}H NMR (DMSO-d_6) δ 6.51(s, 1H), 6.53(s, 1H), 7.09(s, 1H), 7.47(t, 1H, *J* = 7.5 Hz), 7.54(t, 1H, *J* = 7.5 Hz), 8,06(t, 1H), 8.62(s, 1H).

Example 15

Preparation of (5R,6Z)-6-[(7-methoxyimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Step 1: Ethyl 7-methoxyimidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl 7-methoxyimidazo[2,1-b]-benzthiazole-2-carboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from 6-methoxy-2-amino benzothiazole (27 g, 0.15 mol) and ethyl bromopyruvate (39.9 g, 0.2 mol), 24 g (43% Yield) of ethyl 7-methoxyimidazo[2,1-b]-benzthiazole-2-carboxylate was isolated as a brown solid. (M+H) 277.

Step 2: 7-methoxy imidazo[2,1-b]-benzthiazole-2-methanol:

7-methoxy imidazo[2,1-b]-benzthiazole-2-methanol was prepared according to the procedure outlined in Example 1, (Step 2). Starting from ethyl 7-methoxyimidazo[2,1-b]-benzthiazole-2-carboxylate (12.5 g, 43.5 mmol) and $LiAlH_4$ solution (43.5 ml, 0.5 M solution in THF), 4.0 g (40% yield) of the alcohol derivative was isolated as a brown solid. (M+H) 235.

Step 3: 2-Formyl-7-methoxyimidazo[2,1-b]-benzthiazole:

2-Formyl-7-methoxyimidazo[2,1-b]-benzthiazole was prepared according to the procedure outlined in Example 1, (Step 3). Starting from 7-methoxy imidazo[2,1-b]-benzthiazole-2-methanol (4.0 g 17 mmol) in methylene chloride/ DMF(300 mL: 50 mL) and active MnO_2 (12 g, excess), 822 mg (21% Yield) of the aldehyde derivative was isolated as brown solid. (M+H) 233.

Step 4: 4-Nitrobenzyl-6-[(ac tyloxy) (7-methoxyimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-7-methoxyimidazo[2,1-b]-benzthiazole (822 mg, 3.5 mmol) and the dry THF solution (40 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.364, 3.54 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$:etherate (1.3 g, 5mmol) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 2.24 g, 95%; M+H 660.

Step-5: (5*R*),(6*Z*)-6-[(7-methoxyimidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy) (7-methoxyimidazo[2,1-b][1,3]benzothiazol-**2-yl)methyl]-6-**bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (659 mg, 1.0 mmol) was dissolved in THF (17 mL) and acetonitrile (36 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 N HCl was added to adjust pH to 7.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The precipitate was filtered and washed with H_2O, MeCN, acetone to give the title compound. Yield: 68 mg, 23%; as yellow crystals; mp 284; M+H 386.

^1H NMR (DMSO-d_6) δ 3.89 (s, 3H), 6.58(s, 1H), 6.64(s, 1H), 7.14(s, 1H), 7.2(dd,

1H, J = 6.0 Hz), 7.75(d, 1H, J = 3.0 Hz), 8,03(d, J= 6.0 Hz 1H), 8.62(s, 1H).

Example 16

Preparation of (5R,6Z)-6-[(7-chloroimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Ethyl 7-chloroimidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl 7-chloroimidazo[2,1-b]-benzthiazole-2-carboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from 6-chloro-2-amino benzothiazole (9.2 g, 50 mmol) and ethyl bromopyruvate (11.6 g, 60 mmol), 8.5 g (60% Yield) of ethyl 7-chloroimidazo[2,1-b]-benzthiazole-2-carboxylate was isolated as brown solid. (M+H) 281.

Step 2: 7-chloroimidazo[2,1-b]-benzthiazole-2-methanol:

7-chloro imidazo[2,1-b]-benzthiazole-2-methanol was prepared according to the procedure outlined in Example 1, (Step 2). Starting from ethyl 7-chloroimidazo[2,1-b]-benzthiazole-2-carboxylate (9.0 g, 32.1 mmol) and $LiAlH_4$ (4.0 g, excess), 5.5 g (72% yield) of the alcohol derivative was isolated as brown solid. mp 166^{0}C(M+H) 239.

Step 3: 2-Formyl-7-chloroimidazo[2,1-b]-benzthiazole:

2-Formyl-7-chloroimidazo[2,1-b]-benzthiazole was prepared according to the procedure outlined in Example 1, (Step 3). Starting from 7-chloroimidazo[2,1-b]-benzthiazole-2-methanol (4.0 g 16.8mmol) in methylene chloride/ MeOH (300 mL: 50 mL) and active MnO_2 (20 g, excess), 2.2 g (55% yield) of the aldehyde derivative was isolated as brown solid. (M+H) 236.

Step 4: 4-Nitrobenzyl-6-[(acetyloxy) (7-chloroimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-7-chloroimidazo[2,1-b]-benzthiazole (270 mg, 1.14 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (500 mg, 1.14 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was

added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 495 mg, 65%; M+H 665.

Step-5: (5*R*),(6*Z*)-6-[(7-chloroimidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy)(7-chloroimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (450 mg, 0.67 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 80 mg, 18%; as yellow crystals; mp 240^{0}C; (M+H+Na) 412 .

^{1}H NMR (DMSO-d_6) δ 6.6 (s, 2H), 7.1 (s, 1H), 7.62 (dd, 1H), 8.11 (d, 1H), 8.2 (s, 1H), 8.6 (s, 1H).

Example 17

Preparation of (5*R*),(6*Z*)-6-Imidazo[1,2-*a*]quinolin-2-ylmethylene-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Imidazo[1,2-*a*]quinoline-2-carbaldehyde

Imidazo[1,2-*a*]quinoline-2-carbaldehyde was prepared by the method of Westwood and co-workers (*J. Med. Chem.* 1988, *31*, 1098-1115).

Step 1: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxyimidazo[1,2-*a*]quinolin-2-ylmethyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester:

Imidazo[1,2-*a*]quinoline-2-carbaldehyde (1.09 g) and a dry THF solution (75.5 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (2.22 g) were added successively to a dry acetonitrile (75.5 mL) solution of anhydrous $MgBr_2$ (2.5 g) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (1.85 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column, then the column was eluted with $CHCl_3$ -acetone(1/0 ~ 95/5). Collected fractions were concentrated under reduced pressure followed by recrystallization from $CHCl_3$-Et_2O to give the title compound as one isomer. (pale yellow crystals, yield: 1.3 g, 38%).

^{1}H NMR ($CDCl_3$) δ 2.37(s, 3H), 5.29(d, 1H, *J* = 13.5 Hz), 5.45(d, 1H, *J* = 13.5 Hz), 6.22(s, 1H), 7.14(s, 1H), 7.46 ~ 7.52(m, 3H), 7.56(d, 1H, *J* = 9.6 Hz), 7.62(d, 2H, *J* = 8.6 Hz), 7.64 ~ 7.69(m, 1H), 7.83(dd, 1H, *J* = 1.1, 7.9 Hz), 7.93(d, 1H, *J* = 8.3 Hz), 7.99(s, 1H), 8.25(d, 2H, *J* = 8.6 Hz).

Step 2: (5*R*),(6*Z*)-6-Imidazo[1,2-*a*]quinolin-2-ylmethylene-7-oxo-4-thia-1-

azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

(5*R*,6*RS*)-6-[(*RS*)-Acetoxyimidazo[1,2-*a*]quinolin-2-ylmethyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (1.3 g) was dissolved in THF (17 mL) and acetonitrile (36 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 N HCl was added to adjust the pH to 7.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The precipitate was filtered and washed with H_2O, acetonitrile, and acetone to give the title compound, yield 297 mg, 38%, as yellow crystals mp 205°C.

^{1}H NMR (D_2O) d 6.19(s, 1H), 6.36 (s, 1H), 6.87 (s, 1H), 6.96 (d, 1H, *J* = 9.5 Hz), 7.32 (d, 1H, *J* = 9.5 Hz), 7.33 (s, 1H), 7.44 ~ 7.57 m, 4H).

Example 18

Preparation of (5*R*),(6*Z*)-6-(6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of ethyl 6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazole-2-carboxylate;

A mixture of 2-chlorocyclopentanone (11.8 g, 100 mmol) and thiourea (8.0 g 101 mmol) was refluxed in ethanol: THF (1:2) for 16 hrs. The reaction mixture was cooled to room temperature and the separated white solid was filtered. (9.0 g separated) This was dissolved in anhydrous ethanol (100 ml) and sodium methoxide (1.9 g 2.7 g, 51 mmol). To this ethyl bromopyruvate (10 .0 g) was added and stirred at room temperature for 2 hrs. Then it was refluxed for 48 hrs. At the end reaction mixture was cooled to room temperature and concentrated. The residue was extracted with chloroform and washed well with water. The product was purified by silica-gel column chromatography by eluting it with 50% ethyl acetae: hexane. Red semi-solid; Yield: 3.0 g; M+H 237.

The ester was reduced with $LiAlH_4$ and the resultant alcohol was oxidized with active

MnO_2. The aldehyde obtained was taken to next step.

Step 3: Preparation of 4-nitrobenzyl (5R)-6-[(acetyloxy)(6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazol-2-yl)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazole (600 mg, 3.1 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.2 g, 3 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 850 mg, 45%; M+H 620.

Step 4: Preparation of (5*R*),(6*Z*)-6-(6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5R)-6-[(acetyloxy)(6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b][1,3]thiazol-2-yl)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (850 mg, 1.37 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high

vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 138 mg, 29%; as yellow crystals; mp 192^{0}C; (M+H+Na) 346 .^{1}H NMR (DMSO-d_6) δ 8.2 (s, 1H), 7.1 (s, 1H), 6.55 (s, 1H), 6.4 (s, 1H), 3.01 (m, 2H), 2.51 (m, 4H).

Example 19

Preparation of (5*R*),(6*Z*)-6-(Imidazo[1.2-a]quinoxaline-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0] hepto-2-ene-2-carboxylic acid, sodium salt

Imidazo[1,2-a]quinoxaline-2-carboxaldehyde

Imidazo[1,2-a]quinoxaline-2-carboxaldehyde was prepared by the method of Westwood and co-workers (*J. Med. Chem.* **1998**, *31*, 1098-1115).

Step 1: (5R, 6RS)-6-((RS)-Acetoxy imidazo[1,2-a]quinoxalin-2-ylmethyl)-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester:

A dry acetonitrile (33 mL) solution of imidazo[1,2-a]quinoxaline-2-carboxaldehyde (505 mg) was added to a dry acetonitrile (20 mL) solution of $MgBr_2$ (1.1 g) under an nitrogen atmosphere at room temperature, and the mixture was stirred for 10 min. After addition of the dry THF (25 mL) solution of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (931 mg), the mixture was cooled to –20 °C then triethylamine (0.8 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with 4,4-dimethylamino pyridine (58 mg) and acetic anhydride (0.44 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. 10% Citric acid aqueous solution (200 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was

purified by silica gel column chromatography, eluted with CH_2Cl_2 – acetone (50:1), and the title compound was obtained as a diastereomeric mixture (78 : 22, pale brown foamy amorphous, 1.0 g, 68.9%).

^{1}H NMR ($CDCl_3$) δ 2.07 (s, 0.66H), 2.38 (s, 2.34H), 5.30 (d, 1H, *J* = 13.5 Hz), 5.45 (d, 0.78H, *J* = 13.5 Hz), 5.48 (d, 0.22H, *J* = 13.5 Hz), 6.24 (s, 0.78H), 6.46 (s, 0.22H), 6.63 (s, 0.22H), 7.18 (s, 0.78H), 7.50 (s, 0.78H), 7.52 (s, 0.22H), 7.61 (d, 1.56H, *J* = 8.7 Hz), 7.63 (d, 0.44H, *J* = 8.8 Hz), 7.64-7.67 (m, 1H), 7.68-7.73 (m, 1H), 7.92-7.95 (m, 1H), 8.08 (s, 0.78H), 8.13-8.16 (m, 1H), 8.24 (d, 1.56H, *J* = 8.7 Hz), 8.25 (d, 0.44H, *J* = 8.8 Hz), 8.33 (s, 0.22H), 9.05 (s, 0.78H), 9.09 (s, 0.22H).

Step 2: (5*R*),(6*Z*)-6-(Imidazo[1.2-a]quinoxaline-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0] hepto-2-ene-2-carboxylic acid, sodium salt:

(5R, 6RS)-6-((RS)-Acetoxy imidazo[1,2-a]quinoxalin-2-ylmethyl)-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester (951 mg) and 10% Pd-C (50% wet, 477 mg) were added to a mixture of THF (48 mL) and 0.5 mol/L phosphate buffer (pH 6.5, 48 mL). The mixture was hydrogenated at 400 kPa at room temperature for 4 h. The reaction solution was filtered and Pd-C was washed with water and *n*-butanol. The aqueous layer was separated and then the organic layer was extracted with water. The combined aqueous layer was concentrated to 57 g and applied to Diaion HP-21 resin (60 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5, 10, 15 and 20% acetonitrile:water solution (each 60 mL). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid, yield 148 mg (26.1%), mp 300 °C (dec).

^{1}H NMR (D_2O) δ 5.92 (s, 1H), 6.23 (s, 1H), 6.66 (s, 1H), 7.11-7.22 (m, 3H), 7.25 (d, 1H, *J* = 7.9 Hz), 7.50 (s, 1H), 8.03 (s, 1H); IR (KBr) 3413, 1748, 1592, 1553 cm^{-1}; l^{max} (H_2O) 340, 293, 237, 218 nm.

Example 20

Preparation of (5*R*), (6*Z*)-6-(5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Morpholin-3-one

Morpholin-3-one may be prepared in the method of USP 5,349,045.

Step 2: Morpholin-3-thione

A mixture of morpholin-3-one (4.7 g) and Lawesson's reagent (10.3 g) in dry THF (94 mL) was heated to reflux for 1.5 h. The reaction mixture was cooled to room temperature and the reaction solvent was removed in vacuo. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – methanol (50 : 1) to obtain a yellow solid. Recrystallization of the crude product from hexane-ethyl acetate gave the title (4.0 g, 72.2%) as yellow powder.

^{1}H NMR ($CDCl_3$) δ 3.45 (t, 2H, J = 5.1 Hz), 3.91 (t, 2H, J = 5.1 Hz), 4.55 (s, 2H).

Step 3: 5-Methylthio-3,6-dihydro-2*H*-[1,4]oxazine

A mixture of morpholin-3-thione (4.7 g) and methyl iodide (13 mL) in dry CH_2Cl_2 (140 mL) was stirred at room temperature for 15 h. The reaction mixture was filtered and the solid was washed with CH_2Cl_2. The obtained solid was dissolved with 50% K_2CO_3 aqueous solution (150 mL) and the aqueous layer was extracted with CH_2Cl_2 (8 x 100 mL). The combined CH_2Cl_2 layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure and the title was obtained as pale yellow oil (3.6 g, 67.8%).

^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 3.71-3.74 (m, 4H), 4.14-4.15 (m, 2H).

Step 4: 3-Iminomorpholin hydrochloride

A mixture of 5-methylthio-3,6-dihydro-2*H*-[1,4]oxazine (3.6 g) and ammonium chloride (1.5 g) in dry ethanol (136 mL) was heated to reflux for 1 h. The reaction mixture was cooled to room temperature. The reaction solvent was removed in vacuo and the title was obtained as a pale brown solid (3.6 g, 97.7%).

^{1}H NMR (DMSO-d^6) δ 3.34 (m, 2H), 3.86 (t, 2H, J = 5.2 Hz), 4.47 (s, 2H).

Step 5: 5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-2-carbaldehyde (9) and 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-3-carbaldehyde

The mixture of 2-bromo-3-hydroxypropenal (4.1 g), *p*-toluenesulfonic acid

monohydrate (52 mg) and 2-propanol (5.2 mL) in cyclohexane (42 mL) was azeotroped until the vapor temperature rose to 80°C. The reaction mixture was concentrated under reduce pressure. The residue was dissolved in dry ethanol (50 mL). A mixture of the dry ethanol (200 mL) solution of 3-iminomorpholin hydrochloride (3.4 g) and 28% methanol solution of sodium methylate (4.8 g) was added at room temperature. The reaction mixture was stirred at room temperature for 2 h, and then the reaction solvent was removed in vacuo. The residue was dissolved in chloroform (125 mL) and triethylamine (3.5 mL) was added, then the reaction mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and then concentrated under reduce pressure. The residue was dissolved in dichloromethane (300 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduce pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – acetone (4 : 1) to obtain the title (pale orange solid, 1.4 g, 36.3%) and the other regio isomer. (pale orange solid, 609 mg, 16.1%).

Desired product: ^{1}H NMR ($CDCl_3$) δ 4.08-4.15 (m, 4H), 4.88 (s, 2H), 7.58 (s, 1H), 9.85 (s, 1H).

The unwanted regio isomer: ^{1}H NMR ($CDCl_3$) δ 4.06 (t, 2H, *J* = 5.2 Hz), 4.40 (t, 2H, *J* = 5.2 Hz), 4.90 (s, 2H), 7.75 (s, 1H), 9.72 (s, 1H).

Step 6: 5*R*), (6*Z*)-6-(5,6-Dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (66 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazine-2-carbaldehyde (1.2 g) was added to the dry acetonitrile (66 mL) solution of $MgBr_2$ (3.6 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (132 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (3.4 g) was added and the mixture was cooled to –20 °C then triethylamine (2.8 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with 4-dimethylamino pyridine (100 mg) and acetic anhydride (1.5 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 18 h at 0 °C. 10% Citric acid aqueous solution (1 L) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 500 mL). The combined organic layer was washed with

water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (14 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 72 mL) to the THF (72 mL) solution of (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h at room temperature. The reaction solution was filtered through a pad of Celite and the pad was washed with water (170 mL) and *n*-butanol (170 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layer was concentrated to 90 g, 1mol/L NaOH was added to adjust pH to 7.5 and applied to Diaion HP-21 resin (120 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined active fractions was concentrated under high vacuum at 35°C and lyophilized to give the title as a yellow amorphous solid (756 mg, 29.1%).

Mp 130 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.98-4.01 (m, 2H), 4.04-4.07 (m, 2H), 4.74 (AB, 2H, J = 15.3, 22.9 Hz), 6.40 (d, 1H, J = 0.8 Hz), 6.55 (s, 1H), 6.95 (d, 1H, J = 0.6 Hz), 7.54 (s, 1H); IR (KBr) 3412, 1741, 1672, 1592, 1549 cm^{-1}; λ^{max}(H_2O) 304 nm.

Example 21

Preparation of (5*R*),(6*Z*)-6-(5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethyl ester

The titled compound was prepared in the same way of Ranganathan and co-workers (*Indian J. Chem.* **1991**, *30 B*, 169-175).

Step 2: (5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl)methanol

MeOH (2.73 mL) was added to the THF (180 mL) solution of $LiBH_4$ (1.63 g) under a nitrogen atmosphere at room temperature, and then 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethyl ester (8.11 g) was added to the suspension and stirred for 2 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature. Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure to afford the title compound as brown crystals (4.87 g, 78%).

^{1}H NMR ($CDCl_3$) δ 2.44 (t, 1H, *J* = 5.8 Hz), 2.54 – 2.62 (m, 2H), 2.87 (t, 2H, *J* = 7.4 Hz), 4.10 (t, 2H, *J* = 7.2 Hz), 4.63 (d, 2H, *J* = 5.8 Hz), 5.96 (s, 1H).

Step 3: 5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde

MnO_2 (activated) (24.4 g) was added to the $CHCl_3$ (350 mL) solution of (5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl)methanol (4.87 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was reduced under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane – AcOEt (1/1 – 1/2). The title compound was obtained as yellow oil (4.35 g, 91%).

^{1}H NMR ($CDCl_3$) δ 2.63 – 2.71 (m, 2H), 2.95 (t, 2H, *J* = 7.4 Hz), 4.22 (t, 2H, *J* = 7.4 Hz), 6.52 (s, 1H), 9.89 (s, 1H).

Step 4: (5*R*),(6*Z*)-6-(5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde (1.36 g) was added to the dry acetonitrile (148 mL) solution of anhydrous $MgBr_2$ (5.52 g) under a nitrogen atmosphere at room temperature. The dry THF solution (148 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 97%) (3.97 g) was added to the mixture, cooled to –20 °C, and Et_3N (4.18 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with acetic anhydride (1.89 mL) and DMAP (123 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine.

The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (106 mL) and acetonitrile (49 mL). Freshly activated Zn dust (22.5 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 155 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (79 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (848 mg, 29%, pH 7.1).

Mp 190 °C (dec); ^{1}H NMR (D_2O) δ 2.49 (m, 2H), 2.78 (t, 2H, *J* = 7.4 Hz), 4.02 (t, 2H, *J* = 7.4 Hz), 6.01 (s, 1H), 6.29 (s, 1H), 6.90 (s, 2H).

Example 22

Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Tetrahydropyridino[1,2-*c*][1,2,3]oxadiazolone

Conc. HCl (1.96 mL) and $NaNO_2$ (2.2 g) were added to the H_2O (21 mL) solution of *DL*-pipecolic acid (3.04 g) under a nitrogen atmosphere at 0 °C and stirred for 1 h. The solution was extracted with CH_2Cl_2 and organic layer was washed with brine. The mixture was dried over Na_2SO_4 and concentrated under reduced pressure to afford crude (2*RS*)-1-nitrosopiperidine-2-carboxylic acid as pale yellow crystals.

Trifluoroacetic anhydride (1.93 g) was added to the THF (92 mL) solution of crude (2*RS*)-1-nitrosopiperidine-2-carboxylic acid under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C and for 2 h at room temperature. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1/1 – 0/1). The

titled compound was obtained as colorless crystals (1.10 g, 33%).

^{1}H NMR ($CDCl_3$) δ 1.93 – 1.99 (m, 2H), 2.08 – 2.15 (m, 2H), 2.65 (t, 2H, *J* = 6.5 Hz), 4.26 (t, 2H, *J* = 6.1 Hz).

Step 2: 4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxylic acid ethylester

Ethyl propiolate (804 mg) was added to the *o*-xylene (15 mL) solution of tetrahydropyridino[1,2-*c*][1,2,3]oxadiazolone (1.04 g) under a nitrogen atmosphere and refluxed for 16 h. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 – 1/1). The titled compound was obtained as yellow oil (871 mg, 65%), and 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-3-carboxylic acid ethyl ester was obtained as yellow oil (345 mg, 26%).

^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 1.84 – 1.91 (m, 2H), 2.02 – 2.09 (m, 2H), 2.82 (t, 2H, *J* = 6.4 Hz), 4.22 (t, 2H, *J* = 6.2 Hz), 4.39 (q, 2H, *J* = 7.1 Hz), 6.53 (s, 1H).

Step 3: (4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridin-2-yl)methanol

MeOH (0.29 mL) was added to the THF (19 mL) solution of $LiBH_4$ (cont. 90%) (174 mg) under a nitrogen atmosphere at room temperature, then 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxylic acid ethyl ester (862 mg) was added to the suspension and stirred for 1 h at room temperature and 1.5 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature. Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure to afford titled compound as pale yellow oil (691 mg, 95%).

^{1}H NMR ($CDCl_3$) δ 1.80 – 1.87 (m, 2H), 1.98 – 2.05 (m, 2H), 2.77 (t, 2H, *J* = 6.4 Hz), 2.81 – 2.84 (br, 1H), 4.09 (t, 2H, *J* = 6.1 Hz), 4.62 (d, 2H, *J* = 5.3 Hz), 5.96 (s, 1H).

Step 4: 4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carbaldehyde

MnO_2 (activated) (3.36 g) was added to the $CHCl_3$ (44 mL) solution of (4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-yl)methanol (673 mg) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was reduced under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 - 1/2). The titled compound was obtained as pale yellow oil (510 mg, 77%).

^{1}H NMR ($CDCl_3$) δ 1.90 (m, 2H), 2.10 (m, 2H), 2.84 (t, 2H, J = 6.4 Hz), 4.23 (t, 2H, J = 6.2 Hz), 6.52 (s, 1H), 9.92 (s, 1H).

Step 5: (5*R*)(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4,5,6,7-Tetrahydropyrazolo[1,5-*a*]pyridine-2-carbaldehyde (483 mg) was added to the dry acetonitrile (48 mL) solution of anhydrous $MgBr_2$ (1.81 g) under a nitrogen atmosphere at room temperature. The dry THF solution (48 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 97%) (1.28 g) was added to the mixture, cooled to –20 °C, and Et_3N (1.35 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (0.61 mL) and DMAP (40 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (35 mL) and acetonitrile (16 mL). Freshly activated Zn dust (7.43 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 51 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (105 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C

and lyophilized to give the title compound as a yellow amorphous solid (427 mg, 41%, pH 7.7).

Mp 190 °C (dec); ^{1}H NMR (D_2O) δ 1.67 – 1.71 (m, 2H), 1.85 – 1.89 (m, 2H), 2.64 (t, 2H, *J* = 6.3 Hz), 3.97 (t, 2H, *J* = 6.1 Hz), 5.97 (s, 1H), 6.25 (s, 1H), 6.85 (s, 1H), 6.88 (s, 1H).

Example 23

Preparation of (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

Step 1: 5-Methoxy-1-methyl-3,6-dihydro-1H-pyrazin-2-one

The titled compound was prepared in the same way of S.Rajappa and B.G.Advani (*Tetrahedron.* **1973**, *29*, 1299-1302).

Step 2: 5-Amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one

A mixture of 5-methoxy-1-methyl-3,6-dihydro-1H-pyrazin-2-one (2.3 g) and ammonium chloride (936 mg) in dry ethanol (32 mL) was stirred at room temperature for 1 h and then refluxed for 2 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was triturated with chloroform at room temperature for 30 min. The precipitate was filtered off and dried in vacuo. The 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one hydrochloride was obtained as a pale brown powder (1.7 g, 66%).

A solution of 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one hydrochloride (662mg) in methanol (10 mL) was added 10% potassium carbonate aqueous solution at 0°C and then stirred for 40 min at 0°C. The mixture was concentrated under reduced pressure. The residue was triturated with chloroform (18mL) and methanol (2 mL) at room temperature for 30 min. The precipitate was filtered off and dried in vacuo. The compound was obtained as a pale brown powder (515 mg, quantitative).

^{1}H NMR (DMSO-d^6) δ 2.88 (s, 3H), 3.94 (s, 2H), 4.42 (s, 2H).

Step 3: 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde and 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde

The solution of 2-bromo-3-isopropoxy-propenal (1.3 g) in dry acetonitrile (60 mL) was added to the solution of 5-amino-1-methyl-3,6-dihydro-1H-pyrazin-2-one (782 mg) in dry acetonitrile (60 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 h, added triethylamine (0.95 mL) and then refluxed for 2 h. The reaction mixture was cooled to room temperature and then evaporated under reduce pressure. The residue was dissolved in chloroform (10mL) and washed with 50% K_2CO_3 aqueous solution (10mL). The aqueous layer was extracted with chloroform. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduce pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – MeOH (95 : 5) to obtain the title compound 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde as a pale yellow solid (541 mg, 49.1%) and its regio isomer 7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde as a pale yellow solid (128 mg, 11.6%).

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 3.17 (s, 3H), 4.68 (s, 2H), 4.78 (s, 2H) , 7.66 (s, 1H) , 9.83 (s, 1H).

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazine-3-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 3.16 (s, 3H), 4.70 (s, 2H), 5.03 (s, 2H) , 7.82 (s, 1H) , 9.73 (s, 1H).

Step 4: (5*R*, 6*RS*)- 6- [Acetoxy-(7-methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo [1,2-a]pyrazine-2-carbaldehyde (319 mg) was added to the dry acetonitrile (32 mL) solution of anhydrous $MgBr_2$ (786 mg) under a nitrogen atmosphere at room temperature. The dry THF solution (32 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (687 mg) was added to the mixture, cooled to –20 °C, and triethylamine (0.60 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylaminopyridine (44 mg) and acetic anhydride (0.35 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 20 h at 0°C. The mixture was

diluted with ethyl acetate and H_2O. After separating organic layer, the aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with 5% citric acid aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with chloroform. The title compound was obtained as diastereo mixture (yellow amorphous solid ; 410 mg, 38%).

^{1}H NMR (δ, $CDCl_3$) 2.03 (s, 0.7 x 3 H), 2.09 (s, 0.3 x 3H), 3.15 (s, 3H), 4.59-4.62 (m, 2H), 4.66 (s, 0.3 x 2H), 4.67 (s, 0.7 x 2H), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 0.3 x 1H, *J* = 13.5 Hz), 5.45 (d, 0.7 x 1H, *J* = 13.5 Hz), 6.07 (s, 0.3 x 1H), 6.28 (s, 0.7 x 1H), 6.32 (s, 0.7 x 1H), 6.83 (s, 0.3 x 1H), 6.86 (s, 0.3 x 1H), 7.10 (s, 0.7 x 1H), 7.44 (s, 0.3 x 1H), 7.47 (s, 0.7 x 1H), 7.60 (d, 0.7 x 2H, *J* = 8.6 Hz), 7.61 (d, 0.3 x 2H, *J* = 8.6 Hz), 8.24 (d, 2H, *J* = 8.6 Hz),

Step 5: (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt and (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)- 6- [Acetoxy-(7-methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (481 mg) was dissolved in THF (6.7 mL) and acetonitrile (3.1 mL). Freshly activated Zn dust (1.92 g) and 0.5 *M* phosphate buffer (pH 6.5, 9.9 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C and lyophilized. The residue was separated by the preparative HPLC (Inertsil ODS-2, GL Science Inc., 10 x 250 mm, 0.05 mol/L phosphate buffer (pH 7.1) : CH_3CN = 93 : 7, 4.0 mL/min.). The separated fractions of (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt and (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-

carboxylic acid sodium salt were cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0 respectively. Each solution was concentrated under high vacuum at 35 °C. Each concentrate was applied to Diaion HP-21 (60 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5% acetonitrile-water. The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound(5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt as a yellow amorphous solid (125 mg, 44.4 %, Mp 115-117 °C (dec)) and compound (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt as yellow amorphous solid (19 mg, 6.7 %) respectively.

Compound (5*R*),(6*Z*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt ^{1}H NMR (δ, D_2O) 2.99 (s, 3H), 4.54 (s, 2H), 4.66 (s, 2H), 6.38 (s, 1H), 6.85 (s, 1H), 6.90 (s 1H) , 7.30 (s, 1H).

Compound (5*R*),(6*E*)-6-(7-Methyl-6-oxo-5,6,7,8-tetrahydro-imidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt ^{1}H NMR (δ, D_2O) 2.94 (s, 3H), 4.45 (s, 2H), 4.56 (s, 2H), 6.22 (s, 1H), 6.48 (s, 1H), 6.94 (s, 1H), 7.69 (s 1H).

Example 24

Preparation of (5*R*)(6*Z*)-6-(6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

Step 1: (3*R*)-Thiomorpholine-3-carboxylic acid

The titled compound was prepared in the same way of Shiraiwa and co-workers (*Biosci. Biotechnol. Biochem.* **1998**, *62*, 2382-2387).

Step 2: 3-Oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-5-thia-1-aza-7a-azonioindenide

$NaNO_2$ (3.14 g) was added to the 1 mol/L HCl (33.7 mL) solution of (3*R*)-thiomorpholine-3-carboxylic acid (4.96 g) under a nitrogen atmosphere at 0 °C and stirred for 0.5 h. The solution was extracted with $CHCl_3$ (5 times) and the organic layer was washed with brine. The mixture was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude (3*R*)-4-nitrosothiomorpholine-3-carboxylic acid as pale yellow crystals.

Trifluoroacetic anhydride (7.07 g) was added to the THF (169 mL) solution of crude (3*R*)-4-nitrosothiomorpholine-3-carboxylic acid under a nitrogen atmosphere at 0 °C and stirred for 3 h at 0 °C and for 17 h at room temperature. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1/1 – 0/1). The titled compound was obtained as pale brown crystals (3.41 g, 64%).

^{1}H NMR ($CDCl_3$) δ 3.15 (t, 2H, *J* = 5.5 Hz), 3.71 (s, 2H), 4.54 (t, 2H, *J* = 5.5 Hz).

Step 3: 6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carboxylic acid ethyl ester

Ethyl propiolate (2.33 g) was added to the *o*-xylene (72 mL) solution of 3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-5-thia-1-aza-7a-azonioindenide (3.41 g) under a nitrogen atmosphere and refluxed for 15 h. The solution was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (2/1 – 1/1). The titled compound was obtained as yellow oil (3.13 g, 68%), and the other unwanted regio isomer 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-3-carboxylic acid ethyl ester was obtained as yellow oil (556 mg, 12%).

^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, *J* = 7.1 Hz), 3.04 (t, 2H *J* = 5.7 Hz), 3.81 (s, 2H), 4.32 (q, 2H, *J* = 7.1 Hz), 4.40 (t, 2H, *J* = 5.7 Hz), 6.54 (s, 1H).

Step 4: (6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)methanol

$LiBH_4$ (cont. 90%) (536 mg) and MeOH (0.9 mL) was added to the THF (59 mL) solution of 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carboxylic acid ethyl ester (3.13 g) under a nitrogen atmosphere at room temperature and stirred for 3 h at 40 °C. The mixture was quenched with 1 mol/L HCl at pH 1 and stirred for 1 h at room temperature.

Solid K_2CO_3 was added to the solution to adjust pH to 8 and the mixture was extracted with AcOEt. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure to afford titled compound as pale yellow oil (2.51 g, quant.).

^{1}H NMR ($CDCl_3$) δ 2.58 (br, 1H), 3.07 (t, 2H, *J* = 5.7 Hz), 3.84 (s, 2H), 4.33 (t, 2H, *J* = 5.7 Hz), 4.63 (d, 2H, *J* = 3.9 Hz), 6.05 (s, 1H).

Step 5: 6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde

MnO_2 (activated) (11.46 g) was added to the $CHCl_3$ (135 mL) solution of (6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)methanol (2.31 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under a reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1/1). The titled compound was obtained as pale yellow crystals (1.78 g, 78%).

^{1}H NMR ($CDCl_3$) δ 3.15 (t, 2H, *J* = 5.8 Hz), 3.90 (s, 2H), 4.48 (t, 2H, *J* = 5.8 Hz), 6.58 (s, 1H), 9.92 (s, 1H).

Step 6: (5*R*)(6*Z*)-6-(6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (841 mg) was added to the dry acetonitrile (39 mL) solution of anhydrous $MgBr_2$ (1.88 g) under a nitrogen atmosphere at room temperature. The dry THF solution (39 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 99.7%) (1.93 g) was added to the mixture, cooled to –20 °C, and Et_3N (2.79 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (0.94 mL) and DMAP (61 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 17 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure.

The residue was dissolved in THF (83 mL) and acetonitrile (39 mL). Freshly activated Zn dust (7.72 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 122 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (150 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (371 mg, 22%, pH 8.0).

Mp 190 °C (dec); ^{1}H NMR (D_2O) δ 3.03 (t, 2H, *J* = 5.7 Hz), 3.75 (s, 2H), 4.22 (t, 2H, *J* = 5.7 Hz), 6.07 (s, 1H), 6.27 (s, 1H), 6.86 (s, 1H), 6.89 (s, 1H).

Example 25

Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 3-Oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide

Conc. HCl (15 mL) and $NaNO_2$ (16.6 g) were added to the H_2O (166 mL) solution of *L*-thioproline (24.3 g) under a nitrogen atmosphere at 0 °C and stirred for 2 h. The solution was extracted with CH_2Cl_2, organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude N-nitroso derivative as a yellow solid.

Trifluoroacetic anhydride (5.0 mL) was added to the THF (350 mL) solution of crude N-nitrosothioproline under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1 : 1). The titled compound was obtained as a pale brown solid (4.0 g, 15.1%).

^{1}H NMR ($CDCl_3$): δ4.04 (t, 2H, *J* = 1.7 Hz), 5.40 (t, 2H, *J* = 1.7 Hz).

Step 2: **4*H*-5-Thia-1,6a-diazap ntalen-2-carboxylic acid ethyl ster**

Ethyl propiolate (3.1 mL) was added to the *o*-xylene (130 mL) solution of 3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide (4.0 g) under a nitrogen atmosphere and refluxed for 19 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (4 : 1). The titled compound was obtained as a yellow solid (2.7 g, 49.3%), and 4*H*-5-thia-1,6a-diazapentalen-3-carboxylic acid ethylester was obtained as pale yellow crystals (1.2 g, 21.7%).

^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, *J* = 7.1 Hz), 4.11 (d, 2H, *J* = 2.1 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 5.24 (t, 2H, *J* = 1.6 Hz), 6.61 (s, 1H).

Step 3: **(4*H*-5-Thia-1,6a-diazapentalen-2-yl)methanol**

$LiBH_4$ (cont. 90%) (459 mg) was added to the ether (126 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carboxylic acid ethylester (2.5 g) and MeOH (0.77 mL) under a nitrogen atmosphere at room temperature, then refluxed for 1.5 h. The mixture was quenched with 1 mol/L HCl (25 mL) and stirred for 1 h at room temperature. The mixture was neutralized by saturated sodium hydrogen carbonate solution and separated. The aqueous layer was extracted with dichloromethane (10 x 25 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with AcOEt. The titled compound was obtained as a pale yellow solid (1.7 g, 87.9%).

^{1}H NMR ($CDCl_3$) δ 2.95 (t, 1H, *J* = 5.6 Hz), 4.07 (s, 2H), 4.62 (d, 2H, *J* = 5.1 Hz), 5.13 (t, 1H, *J* = 1.6 Hz), 6.04 (s, 1H).

Step 4: **4*H*-5-Thia-1,6a-diazapentalen-2-carbaldehyde**

The dry dichloromethane (8 mL) solution of dimethylsulfoxide (2.2 mL) was added dropwise to the dry dichloromethane (110 mL) solution of oxalyl chloride (2.0 mL)

at -78°C. The reaction mixture was stirred for 15 min at the same temperature. The dry dichloromethane (40 mL) solution of (4*H*-5-thia-1,6a-diazapentalen-2-yl)methanol, (1.7 g) was added dropwise to the reaction mixture at -78°C, and stirring was continued for an additional 15 min. The reaction mixture was allowed to warm to -45°C and stirred for 1 h. Triethylamine (11.3 mL) was added dropwise and the reaction mixture was allowed to warm to 0°C. After 20min, saturated ammonium chloride solution (50 mL) and water (100 mL) were added and separated. The aqueous layer was extracted with AcOEt (3 x 150 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with hexane – AcOEt (1 : 1). The titled compound was obtained as a yellow solid (1.7 g, quant.).

^{1}H NMR ($CDCl_3$) δ 4.13 (s, 2H), 5.26 (d, 2H, J = 1.4 Hz), 6.59 (s, 1H), 9.90 (s, 1H).

Step 5: (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (92 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carbaldehyde (1.7 g) was added to the dry acetinitrile (92 mL) solution of $MgBr_2$ (5.0 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (184 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (4.3 g) was added and the mixture was cooled to –20 °C then triethylamine (7.4 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (138 mg) and acetic anhydride (2.1 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The 1mol/L Citric acid aqueous solution (1000 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 400 mL). The combined organic layers were washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as a brown amorphous solid.

Freshly activated Zn dust (19.3 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 100 mL) to the THF (100 mL) solution of crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h at room temperature. The reaction solution was filtered through a pad of Celite and the pad was washed with water (200 mL) and *n*-butanol (200 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layers were concentrated to 90 g, 1mol/L NaOH was added to adjust pH to 8.0 and applied to Diaion HP-21 resin (180 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 15% acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid (634 mg, 17.4%, pH 7.25).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ4.00 (s, 2H), 5.09 (s, 2H), 6.14 (s, 1H), 6.36 (s, 1H), 6.91 (s, 1H), 6.92 (s, 1H); IR (KBr) 3381, 1752, 1683, 1600, 1558 cm^{-1}; λ^{max} (H_2O) 292, 196 nm.

Example 26

Preparation of (5*R*)(6*Z*)-6-(7*H*-Imidazo[1,2-c]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Thiazolidin-4-one

The titled compound was prepared in the same way of Marvin M. and Allen R. Harkness. (*Tetrahedron Letters.* **1994**, *35*, 6971-6974).

Step 2: Thiazolidine-4-thione

Lawesson's reagents (33.5 g) added to the solution of thiazolidin-4-one (14.2 g) in dry THF (690 mL) and the reaction mixture was refluxed for 2 h. The mixture was cooled to room temperature and evaporated under reduced pressure. The residue was triturated with $CHCl_3$:MeOH=7:3 solution (65 mL) at room temperature for 30 min. The

precipitate was filtered off, washed with $CHCl_3$:*n*-hexane=7:3 solution (15 mL) and dried in vacuo. The thiazolidine-4-thione was obtained as a pale yellow powder (10.7 g, 65%).

^1H NMR ($CDCl_3$) δ 4.08 (s, 2H), 4.70 (s, 2H).

Step 3: 4-Methylthio-2,5-dihydro-thiazole

Methyl iodide (28.4 g) was added to the boiling solution of thiazolidine-4-thione (9.5 g) in chloroform (400 mL), and the reaction mixture was refluxed for 1.5 h. To the reaction mixture, an additional methyl iodide (56.8 g) was added in 5 portions at 30-60 min intervals. After refluxing for additional 1 h, the reaction mixture was cooled to room temperature. Then 10% potassium carbonate aqueous solution (200 mL) was added and stirred for 15min at room temperature. After separating organic layer, the aqueous layer was extracted with $CHCl_3$ (100 mL x 3). Organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and dried in vacuo. After drying, the title compound was obtained as brown oil (11.0 g, quant.).

^1H NMR ($CDCl_3$) δ 2.51 (s, 3H), 3.91 (t, 2H, J = 3.5 Hz), 5.21 (t, 2H, J = 3.5 Hz).

Step 4: Thiazolidin-4-ylideneamine

A mixture of 4-methylthio-2,5-dihydrothiazole (10.7 g) and ammonium chloride (6.4 g) in dry ethanol (400 mL) was refluxed for 27.5 h. The reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was dissolved in chloroform (300 mL) and 10% potassium carbonate aqueous solution (200 mL), then stirred for 20 min at room temperature. After separating organic layer, the aqueous layer was extracted with chloroform (100 mL x 5). Organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and dried in vacuo to obtain crude thiazolidin-4-ylideneamine (5.5g) as a brown solid that included by product, which is an ethoxy derivative and 4-methylthio-2,5-dihydrothiazole, which is the starting material . The ratio of these three compounds was determined to be 61:34:5 respectively by ^1H-NMR.

^1H NMR ($CDCl_3$) δ 3.75 (t, 2H, J = 2.8 Hz), 4.97 (t, 2H, J = 2.9 Hz).

Step 5: ***7H*-Imidazo[1,2-c]thiazole-2-carbaldehyde**

The solution of 2-bromo-3-isopropoxypropenal (6.9 g) in dry acetonitrile (326 mL) was added to the solution of crude thiazolidin-4-ylideneamine (3.3 g) in dry acetonitrile (326 mL) at room temperature. The reaction mixture was stirred at room temperature for 19.5 h, added triethylamine (4.9 mL) and then refluxed for 2 h. The reaction mixture was cooled to room temperature and then evaporated under reduce pressure. The residue was dissolved in dichloromethane (300 mL) and washed with 50% potassium carbonate aqueous solution (20 g). After filtration and separation, the aqueous layer was extracted with dichloromethane (50 mL x 4). The organic layers were combined, dried ($MgSO_4$) and filtered. The filtrate was evaporated under reduced pressure. The residue was applied to silica gel column chromatography and eluted with $CHCl_3$ – MeOH (100 : 3) to obtain crude 7*H*-Imidazo[1,2-c]thiazole-2-carbaldehyde as a brown solid. The crude product was re-crystallized twice from $CHCl_3$ – *n*-hexane (1^{st}: 30:5, 2^{nd}: 30:60) at 0 °C to give the required aldehyde as pale brown crystals (Yield: 1.84 g, 15 %).

^{1}H NMR ($CDCl_3$) δ 4.09 (t, 2H, *J* = 1.3 Hz), 5.08 (t, 2H, *J* = 1.2 Hz), 7.63 (s, 1H), 9.81 (s, 1H).

Step 6: **(5*R*)(6*Z*)-6-(7*H*-Imidazo[1,2-c]thiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid, sodium salt**

7*H*-Imidazo[1,2-c]thiazole-2-carbaldehyde (841 mg) was added to the dry acetonitrile (116 mL) solution of anhydrous $MgBr_2$ (2.93 g) under a nitrogen atmosphere at room temperature. The dry THF solution (116 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 99.7%) (2.51 g) was added to the mixture, cooled to –20 °C, and Et_3N (2.20 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 4 h at –20 °C and treated with acetic anhydride (1.26 mL) and DMAP (160 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under

reduced pressure.

The residue was dissolved in THF (53 mL) and acetonitrile (25 mL). Freshly activated Zn dust (15.1 g) and 0.5 *M* phosphate buffer (pH 6.5, 78 mL) were added to the mixture. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (321 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (1.1 g, 51%, pH 7.5).

Mp 145 °C (dec); ^{1}H NMR (D_2O) δ 3.85 (s, 2H), 4.88 (s, 2H), 6.32 (s, 1H), 6.78 (s, 1H), 6.85 (s, 1H), 7.27 (s, 1H).

Example 27

Preparation of (5R,6Z)-7-oxo-6-[(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methylene]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid.

Step 1: Diethyl 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarboxylate

To a solution of diethyl 3,5-pyrazoledicarboxylate (2.17 g, 10 mmol) in acetonitrile (10 ml), under nitrogen, was added potassium carbonate (2.07 g, 15 mmol), and 2-bromoethoxy-t-butyldimethylsilane (2.90 g, 12 mmol). The mixture was stirred at reflux for 18 hr. It was then cooled to room temperature, diluted with ethyl acetate (20 ml), and filtered through Magnesol. The filter pad was eluted wtih 2 x 10 ml of ethyl acetate, and the combined filtrate was evaporated. The residue was dissolved in hexanes and passed through a column of silica gel (70 g). After eluting with hexanes (100 ml), the column was eluted with ethyl acetate. The ethyl acetate eluent was evaporated to give 3.71 g of a colorless oil; MS m/e 371 (MH^+).

Step 2: 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dim thanol

To a solution of diethyl 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarboxylate (0.74 g, 2 mmol) in methylene chloride (8 ml), under nitrogen, was added 12 ml of a 1.0 M solution of diisobutylaluminum hydride in methylene chloride at 0 °C. After stirring at 0 °C for 0.5 hr, the mixture waas warmed to room temperature for 0.5 hr. It was then quenched with 15 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic extract was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give 0.44 g of a white solid; mp 82-83 °C; MS m/e 287 (MH^+).

Step 3: 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarbaldehyde

To a stirred solution of 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dimethanol (1.18 g, 4 mmol) in methylene chloride (20 ml), was added 4-methylmorpholine-N-oxide (2.89 g, 24 mmol) and molecular sieve 4A (4 g). The reaction mixture was stirred at room temperature for 10 min. and then treated with tetrapropylammonium peruthenate (0.15 g, 0.4 mmol). Stirring was continued for 2 hr. The methylene chloride solution was concentrated and diluted with ether (40 ml). The mixture was filtered through a pad of silica gel (40 g) and the filter pad was eluted with 2 x 20 ml ether. The combined eluent was washed with 1N HCl and brine, dried over anhydrous sodium sulfate, and evaporated to give 0.79 g of a white solid; mp 63-64 °C; MS m/e 283 (MH^+).

Step 4: 4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde

To a solution of 1-(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)-1H-pyrazole-3,5-dicarbaldehyde (1.02 g, 6.07 mmol) in THF (30 ml) was added 6.68 ml of a 1.0 M solution of tetrabutylammonium fluoride in THF at 0 °C. After stirring for 1 hr, the mixture was treated with 10 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered through Magnesol and evaporated. The crude gum was washed with hexanes, dried in vacuo, and then dissolved in methylene chloride (20 ml). To this

solution was added 4-methylmorpholine-N-oxide (2.89 g, 24 mmol) and molecular sieve 4A (6 g). The mixture was stirred at room temperature for 10 min. and then treated with tetrapropylammonium peruthenate (0.11 g, 0.3 mmol). Stirring was continued for 2 hr. The methylene chloride solution was concentrated and diluted with ethyl acetate (40 ml). The mixture was filtered through a pad of silica gel (40 g) and the filter pad was eluted with 2 x 20 ml ethyl acetate. The combined eluent was washed with 1N HCl and brine, dried over anhydrous sodium sulfate, and evaporated to give 0.30 g of a white solid; mp 135-136 °C; MS m/e 167 (MH^+).

Step 5: 4-nitrobenzyl (5R)-6-[(acetyloxy)(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

To a solution of $MgBr_2$ (0.46 g, 2.52 mmol) in acetonitrile (13 ml) under nitrogen was added 4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde (0.14 g, 0.84 mmol) at room temperature with stirring. A solution of (5R,6S)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (0.32 g, 0.84 mmole) in THF (13 ml) was then added, and the mixture was cooled to –20 °C. Triethylamine (0.35 ml, 2.52 mmol) was introduced, and the mixture was stirred at –20 °C in the dark for 4 hr. It was then treated with acetic anhydride (0.2 ml, 2.0 mmol), and 4-N,N-dimethylaminopyridine (12 mg, 0.1 mmol), and kept at 0 °C for 18 hr. The mixture was concentrated and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with 5% citric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The crude material was chromatographed with silica gel (EtOAc-CH2Cl2/1:5) to give 0.27 g of an off-white solid; mp 107-110 °C; MS m/e 595 (MH^+).

Step 6: (5R,6Z)-7-oxo-6-[(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methylene]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid

To a solution of 4-nitrobenzyl (5R)-6-[(acetyloxy)(4-oxo-6,7-dihydro-4H-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.22 g, 0.37 mmol) in THF (15 ml), under nitrogen, was added 15 ml of a

phosphate buffer solution (0.5M, pH 6.5), and 80 mg of 10% Pd/C. The mixture was hydrogenated at 40-50 psi for3 hr, and then filtered through Celite. The filter pad was washed with THF, and the filtrate was extracted with ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was washed with ether to give 0.07 g of a yellow solid; MS m/e 320 (MH^+); ^1H NMR (DMSO-d_6) δ 4.55-4.57 (m, 2H), 4.76-4.80 (m, 2H), 6.50 (s, 1H), 6.63 (s, 1H), 7.58 (s, 1H), 7.76 (s, 1H).

Example 28

Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]pyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 6,7-Dihydro-4H-thieno[3,2-c]pyran-2-carbaldehyde

POCl3 (3.83ml, 50mmol) was added dropwise to ice cooled DMF (3.85ml, 50mmol) within 3 minutes. DCM (20ml) was added and the bath was removed when the reaction media appeared to be pasty. The reaction was kept at 23oC for 2 hrs. Then it was cooled to 0oC again. 4H-pyran-4-one (5 gram, 50mmol) in 10ml DCM was then added dropwise within 3 minutes. The reaction was kept at 0oC for 2 hrs. Pour the mixture onto ice and sodium acetate solution and extract with DCM (2x200). The combined organic layers were dried over magnesium sulfate. Filter off the drying agent and concentrate gave 5.0 gram of product. The compound was dissolved in DCM (200ml) and was added 6.0 gram of ethyl 2-6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carbaldehyde-acetate and 10 ml TEA. The mixture was refluxed for 18 hrs. Then it was washed with water and dried over magnesium sulfate. It was then filtered, concentrated and flash chromatographed with 20 ethyl acetate in hexane. The collected material was dissolved in 100ml THF and LAH (150ml, 0.5M in THF) was injected and left at 23oC for 10 minutes. Then it was refluxed for 18 hrs. Quenched at 23oC by adding water and eventually 1N HCl to clear up the mixture. Extract with ethyl acetate (2x200ml) and combined organic layers dried over magnesium sulfate. Filter and concentrate gave 2.3gram product. The crude material was dissolved in DCM (300ml) and manganese dioxide (15 gram was added). The reaction was carried on at 23oC for 0.5 hr. Then 2x15 gram of oxidant was added each half an hour later. The material was then filtered through a pad of celite concentrated. Flash column chromatography gave 1.206gram

(14% yield) oil product.

H-NMR:δ 9.84(s, 1H), 7.41(s, 1H), 4.74 (s, 2H), 4.00 (t, 2H, J=5.6 Hz), 2.96 (t, 2H, J=5.6Hz); MS: 169.1(M+H)

Step 2: Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]pyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7-Dihydro-4H-thieno[3,2-c]pyran-2-carbaldehyde (336mg, 2mmol) was dissolved in 20ml acetonitrile and magnesium bromide (516 mg, 2mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770mg, 2mmol) in 20ml THF was then injected all at once and the mixture was immediately cooled to –20oC/ Triethylamine (1ml) was then injected and the mixture stirred at –20oC for three hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 18 hrs. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 491mg (41%) product. This product was then dissolved in 15ml THF and 15ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 45psi hydrogen for one hour with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 100 mg (38%) of product was collected; MP: >250^0 C;

H-NMR: δ 7.36 (s, 1H), 7.15(s, 1H), 6.55(s, 1H), 6.44(s, 1H), 4.61 (s, 2H), 3.88(m, 2H), 2.86 (m, 2H), 2.27 (m, 2H), 1.43 (t, 3H)

MS: 320.3(M-H)

Example 29

Preparation of 6-(6,7-Dihydro-4H-thi no[3,2-c]thiopyran-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carbaldehyde

POCl3 (4.02ml, 43mmol) was added dropwise to ice cooled DMF (3.34ml, 43mmol) within 3 minutes. DCM (20ml) was added and the bath was removed when the reaction media appeared to be pasty. The reaction was kept at 23oC for 2 hrs. Then it was cooled to 0oC again. Tetrahydro-thiopyran-4-one (5 gram, 43mmol) in 10ml DCM was then added dropwise within 3 minutes. The reaction was kept at 0oC for 2 hrs. Dilute with DCM (250 ml) and then wash with ice cold 200ml saturated sodium acetate aqueous solution. The organic layer was dried over sodium sulfate. Filter off the drying agent, concentrate and flash column chromatography using 10% ethyl acetate in hexane gave 1.3 gram (8mmol) of product. The compound was dissolved in DCM (100ml) and was added 1.2ml (11 mmol) of ethyl 2-mercapto-acetate and 1ml TEA. The mixture was refluxed for 18 hrs. Then it was washed with water and dried over magnesium sulfate. Filter, concentrate and flash chromatograph with 20 ethyl acetate in hexane produced 1.1gram (11% yield) of product

H-NMR:δ 6.68(s, 1H), 4.73 (s, 2H), 3.68(s, 2H), 3.04 (t, 2H, J=7.6 Hz), 2.91 (t, 2H, J=7.6Hz).; MS (EI): 185.99 (M+)

The 1.1 gram (4.8mmol) 6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carboxylic acid ethyl ester was dissolved in 100ml THF and LAH (40ml, 0.5M in DMG) was injected and the reaction was left at 23oC for 10 minutes. Then it was refluxed for 18 hrs. Quenched at 23oC with water (10ml). The organic layer decanted and the remaining was washed with 20ml DCM. The combined organic layers dried over sodium sulfate. Filter, concentrate and flash column chromatograph with 10-20% ethyl acetate produced 940mg crude product. This crude material was dissolved in DCM (40ml) and manganese dioxide (2 gram was added). The reaction was carried on at 23oC for half an hour. The material was then filtered through a pad of celite concentrated. Flash column chromatography gave 320mg (36%) product.

H-NMR:δ 9.82(s, 1H), 7.46 (s, 1H), 3.56 (s, 2H), 3.15 (t, 2H, J=7.2 Hz), 2.95 (t, 2H, J=7.2 Hz).; MS (EI): 228.02 (M+)

Step 2: Preparation of 6-(6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-ylmethylene)-7-

oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7-Dihydro-4H-thieno[3,2-c]thiopyran-2-carbaldehyde (320mg, 1.72mmol) was dissolved in 17ml acetonitrile and magnesium bromide etherate (450 mg, 1.74mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (660mg, 1.72mmol) in 17ml THF was then injected all at once and the mixture was immediately cooled to –20oC/ Triethylamine (1ml) was then injected and the mixture stirred at –20oC for three hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 18 hrs. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 461mg (44%) product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for one hour and half with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 21 mg (8.6%) of product was collected.

MP: >250^0 C

H-NMR: 7.34 (s, 1H), 7.18(s, 1H), 6.59(s, 1H), 6.44(s, 1H), 3.71 (s, 2H), 2.93(s, 2H), 2.50 (s, 2H).; MS: 338.0(M+H)

Example 30

Preparation of 6-(5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of (5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-yl)-methanol

6,7-Dihydro-4H-thieno[3,2-c]pyridine-2,5-dicarboxylic acid diethyl ester (46 gram, 163mmol) was dissolved in 200ml THF. The solution was injected LAH (1M, THF) 300ml at 23oC. Then it was stirred at 23oC for 18 hrs. The reaction was quenched with

10ml water and dried directly over sodium sulfate. Filter and concentrate yielded 29.3 gram (160mmol, 98%) crude product.
H-NMR: 6.55(s, 1H), 4.70 (s, 2H), 3.41 (s, 2H), 2.86 (t, 2H, J=5.6 Hz), 2.73 (t, 2H, J=5.6 Hz), 2.38 (s, 3H); MS: 184.0(M+H)

Step 2: Preparation of 5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-2-carbaldehyde

DMSO (1.7ml, 24mmol) in 5ml CH_2Cl_2 was cooled to –50-60oC. Oxalyl chloride (1ml, 11mmol) in 20ml DCM was then added within 5 minutes at 50oC. The mixture was kept at –50oC for 5 minutes and then 1.67 gram (9mmol) of (5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-yl)-methanol in 20ml DCM was added at 50oC and the mixture was stirred for another 15minutes at 50oC. Triethylamine (7ml) was then added at –50oC and after 5 minutes the bath was removed and the mixture is naturally warmed up to 23oC. It was washed with 100ml water and extracted with 100ml ethyl acetate. The combined organic layers were dried over magnesium sulfate. Filter. Concentrate and flash column chromatograph using 0-15% methanol in ethyl acetate yielded 736mg (45% yield) product.
H-NMR: 9.81(s, 1H), 7.42 (s, 1H), 3.56 (s, 2H), 3.00 (t, 2H, J=5.6 Hz), 2.91 (t, 2H, J=5.6 Hz), 2.51 (s, 3H); MS: 182.1(M+H)

Step 3: Preparation of 6-(5-Methyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridin-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

2-formyl-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester (724mg, 4mmol) was dissolved in 40ml acetonitrile and magnesium bromide etherate (1.2 gram, 4.65mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54gram, 4mmol) in 40ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (2ml) was then injected and the mixture stirred at –20oC for 3 hrs. Then acetic anhydride (0.66ml) was injected and the mixture was stirred at 0oC for 48 hrs. The reaction media was then diluted with 500ml ethyl acetate and washed with 50 ml 5% citric acid, 50 ml saturated sodium bicarbonate, and 50ml brine. Another 300ml ethyl acetate was used to wash each

aqueous solution. The combined organic layers were then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 1.56 gram (64% yield) product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for two hrs with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 112 mg (13%) of product was collected.

MP: >250oC

H-NMR: δ 7.48 (s, 1H), 7.37(s, 1H), 7.21(s, 1H), 7.10(s, 1H), 3.41(s, 2H), 2.88 (s, 2H), 2.68(s, 2H), 2.37(s, 3H); MS: 335.0(M+H)

Example 31

Preparation of 2-(2-Carboxy-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-en-6-ylidenemethyl)-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester

2-Formyl-6,7-dihydro-4H-thieno[3,2-c]pyridine-5-carboxylic acid ethyl ester (480mg, 2mmol) was dissolved in 20ml acetonitrile and magnesium bromide etherate (516mg, 2mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770mg, 2mmol) in 20ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (1ml) was then injected and the mixture stirred at –20oC for 3 hrs. Then acetic anhydride (0.4ml) was injected and the mixture was stirred at 0oC for 48 hrs. The reaction media was then diluted with 200ml ethyl acetate and washed sequentially with 50 ml 5% citric acid, 50 ml saturated sodium bicarbonate, and 50ml brine. The organic layer was then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 690mg (50%, yield) product. A fraction of this product (456mg, 0.69mmol) was then dissolved in 15ml THF and 15ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for two hrs with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove

most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 18 mg (5%) of product was collected.

MP: >250oC

H-NMR: 7.35 (s, 1H), 7.24 (s, 1H), 6.61 (s, 1H), 6.45(s, 1H), 4.48 (s, 2H), 4.08 (quartet, 2H, J=7.2Hz), 3.68 (m, 2H), 2.87(m, 2H), 1.20 (t, 3H, J=7.2Hz); MS: 393.0(M+H)

Example 32

Preparation of 7-Oxo-6-(6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 6,7,8,9-Tetrahydro-5H-imidazo[1,2-a]azepine-2-carbaldehyde

Thiocaprolactam (6.45 gram, 50mmol) was dissolved in 400ml CH_2Cl_2 and methyl iodide (16ml, 5eq) was next added. The mixture was stirred under nitrogen for 18 hrs. Then it was treated with 100ml potassium carbonate (50%, aq.). The organic layer was then dried over magnesium sulfate. After filtration and concentration 7.3 gram of material was obtained. This material was dissolved 300ml ethanol and 2.83 gram of ammonium chloride was added. The mixture was refluxed for 1 hr. Then the solvent was removed in vacuo. Half of the material was added 200ml ethanol and then followed by addition of 1.35gram (25mmol) sodium methoxide and 4.8gram (25mmol) 2-Bromo-3-isopropoxy-propenal and the mixture was stirred at 23oC for 2 hrs. Then the solvent was removed and 200ml chloroform was added along with 10ml triethyl amine. The mixture was refluxed for 2 hrs and then cooled to 23oC. The reaction media was partitioned between 300ml DCM and 2x150 potassium carbonate (50%). The organic layer was dried over magnesium sulfate. After filtration and concentration 2.1gram of oil product was obtained.

H-NMR: 9.62 (s, 1H), 7.60 (s, 1H), 6.61 (s, 1H), 6.45(s, 1H), 4.58 (s, 2H), 2.96 (2m, H), 1.90(m, 2H), 1.72 (m, 2H); MS: 164.9(M+H)

Step 2: Preparation of 7-Oxo-6-(6,7,8,9-tetrahydro-5H-imidazo[1,2-a]azepin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6,7,8,9-Tetrahydro-5H-imidazo[1,2-a]azepine-2-carbaldehyde (1.312gram, 8mmol) was dissolved in 80ml acetonitrile and magnesium bromide etherate (2.94gram, 8mmol) was then added under N2 atmosphere. The mixture was stirred at 23oC for half an hour. 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.155gram, 3mmol) in 60ml THF was then injected all at once and the mixture was immediately cooled to –20oC. Triethylamine (4ml) was then injected and the mixture stirred at –20oC for 4 hrs. Then acetic anhydride (1ml) was injected and the mixture was stirred at 0oC for 20 hrs. The reaction media was then diluted with 500ml ethyl acetate and washed with 100ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over sodium sulfate. Filter, concentrate, and flash column chromatograph using 20% ethyl acetate in hexane gave 800mg product. This product was then dissolved in 20ml THF and 20ml aqueous phosphate buffer (pH=6.5). The mixture was then subjected to 40psi hydrogen for 1 hr with 0.5gram 10% palladium on carbon. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 2 liter of water followed by 5% acetonitrile in water. Water was then removed through concentrate in vacuo and 131 mg (31%) of product was collected.
MP: >250^0 C
H-NMR: δ 7.78 (s, 1H), 7.02 (s, 1H), 6.94 (s, 1H), 6.36 (s, 1H), 3.92(m, 2H), 2.80 (m, 2H), 1.78 (m, 2H), 1.61(m, 2H), 1.54(m, 2H); MS: 318.2(M+H).

Example 33

(5*R*),(6*Z*)-6-(7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester

Et_3N (6.27 mL), PhCHO (4.92 mL) were added successively to the EtOH (81 mL) solution of 5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester, hydrochloride (9.47 g) at room temperature and stirred for 3 h under a nitrogen atmosphere. Then $NaBH_3CN$ (2.97 g) was added to the reaction mixture and stirred for

19 h. The mixture was filtered through a pad of Celite and diluted with CH_2Cl_2 and washed with 50% K_2CO_3 aq. The organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (1/0 ~ 9/1) and $CHCl_3$ - MeOH (19/1 ~ 9/1). The titled compound was obtained as pale yellow crystals (4.16 g, 36%).

^{1}H NMR($CDCl_3$) δ 1.36(t, 3H, *J* = 7.1 Hz), 2.87(t, 2H, *J* = 5.2 Hz), 3.71(s, 2H), 3.75(s, 2H), 4.01(m, 2H), 4.34(q, 2H, *J* = 7.1 Hz), 7.25-7.34(m, 5H), 7.51(s, 1H) .

Step 2: 7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde

1.01 *M* solution of DIBAL in toluene (1 mL + 0.2 mL + 0.3 mL) was added to the dry CH_2Cl_2 (5 mL) solution of 7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carboxylic acid ethyl ester (283 mg) under a nitrogen atmosphere at –78 °C and stirred for 1.5 h. The mixture was quenched with 1*M* HCl (5 mL). The reaction mixture was filtered through a pad of Celite. The filtrate was washed with 50% K_2CO_3 aq. and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried (K_2CO_3) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with $CHCl_3$ - acetone (9/1 ~ 4/1) and $CHCl_3$ - MeOH (19/1). The titled compound was obtained as colorless crystals (148 mg, 61%).

^{1}H NMR($CDCl_3$) δ 2.90(t, 2H, *J* = 5.5 Hz), 3.74(s, 2H), 3.76(s, 2H), 4.06(t, 2H, *J* = 5.5 Hz), 7.28 ~ 7.35(m, 5H), 7.53(s, 1H), 9.80(s, 1H).

Step 3: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (diastereo mixture)

7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine-2-carbaldehyde (139 mg) was added to the dry acetonitrile (8.7 mL) solution of anhydrous $MgBr_2$ (325 mg) under a nitrogen atmosphere at room temperature. The dry THF solution (8.7 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (223 mg) was added to the mixture, cooled to –20 °C, and Et_3N (0.24 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 5 h at –20 °C and treated with acetic anhydride (0.11

mL) and DMAP (7 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (3/1 ~ 1/1). The titled compound was obtained as two diastereo mixture (80/20, purple amorphous solid, 233 mg, 61%).

^{1}H NMR($CDCl_3$) δ 1.99(s, 0.8x3H), 2.23(s, 0.2x3H), 2.83 ~ 2.89(m, 2H), 3.68(d, 2H, *J* = 4.9 Hz), 3.71(s, 2H), 3.94 ~ 4.13(m, 2H), 5.27(d, 1H, *J* = 13.6 Hz), 5.41(d, 0.2x1H, *J* = 13.6 Hz), 5.45(d, 0.8x1H, *J* = 13.6 Hz), 6.05(s, 0.2x1H), 6.28(s. 0.8x1H), 6.31(s, 0.8x1H), 6.790(s, 0.2x1H), 6.793(s, 0.2x1H), 7.01(s, 0.8x1H), 7.27 ~ 7.36(m, 5H), 7.42(s, 0.2x1H), 7.46(s, 0.8x1H), 7.61(d, 2H, *J* = 8.6 Hz), 8.22(d, 2H, *J* = 8.6 Hz).

Step 4: (5*R*),(6*Z*)-6-(7-Benzyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(7-benzyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazin-2-yl) methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl
ester (1.27 g) was dissolved in THF (55 mL) and acetonitrile (25 mL). Freshly activated Zn dust (5.08 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 80 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (79 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN(1/0 ~ 4/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (390 mg, 49%, pH 7.7).

Mp 180 °C (dec); ^{1}H NMR(D_2O) δ 2.84 ~ 2.95(m, 2H), 3.61(d, 2H, *J* = 7.2 Hz),

3.67(s, 2H), 3.96(t, 2H, *J* = 5.7 Hz), 6.43(s, 1H), 6.89(s, 1H), 6.93(s, 1H), 7.28 ~7.37(m, 6H).

Example 34

Preparation of (5R,6Z)-7-oxo-6-{[5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (1.05 g, 5.2 mmol) in DMF (20 ml) , 3-picolyl chloride hydrochloride (0.852 g, 5.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow semi-solid. Yield: 800 mg , 59%; M+H 259.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (516 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The

organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 700 mg, 51%; M+H 685 and 687.

Step 3: (5R,6Z)-6-{[5-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylmethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (686 mg, 1.0 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 12%; as yellow crystals; mp. 134-136^{0}C; (M+H) 412 .

^{1}H NMR (DMSO-d_6)δ d 2.8 (m, 2H), 2.92 (bm, 2H), 3.6 (m, 2H), 3.86 (s, 2H), 6.3 (s, 1H), 6.41 (s, 1H), 7.17 (s, 1H), 7.29 (s, 1H), 7.35 (m, 1H), 7.7 (m, 1H), 8.48 (d,1H), 8.54 (s, 1H).

Example 35

Preparation of (5R,6Z)-7-oxo-6-{[5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (606 mg, 3.0 mmol) in DMF (20 ml) , nicotinoyl chloride hydrochloride (531 mg, 3.0 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. Pale yellow semi-solid. Yield: 600mg , 73%; M+H 273.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)carbonyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (400 mg, 1.4 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (619 mg, 2.4 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 300 mg, 30%; M.pt. 71^{0}C; M+H 701.

Step 3: (5R,6Z)-6-{[5-(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium

salt:

4-Nitrobenzy-6-[(acetyloxy)[5(pyridin-3-ylcarbonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (800 mg, 1.14 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% CAN: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 50 mg, 12%; as yellow crystals; mp. 195 ^{0}C; (M+H) 426.

Example 36

Preparation of (5R,6Z)-7-oxo-6-{[5-(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 2-Formyl [5-(phenylacetyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine:

To a stirred solution of 2-(formyl)-6,7-dihydrothieno[3,2-c]-5(4H)-pyridine (0.41 mg, 2 mmol) in DMF (20 ml) , phenyl acetyl chloride (0.35 mg, 2.2 mmol) and N,N-diisopropylethylamine (10 ml, excess) was added at room temperature. The reaction mixture was stirred for 24 hrs and quenched with water. The reaction mixture was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The product was purified by SiO_2 column chromatography by eluting it with ethylacetate. White solid. Yield: 510 mg , 89%; M+H 286.

Step 2: 4-Nitrobenzy-6-[(acetyloxy)[5(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl [5-(phenylacetyl)-4,5,6,7-tetrahydrothieno][3,2-c]pyridine (340 mg, 1.2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (390 mg, 1.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (310 mg, 1.2 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 360 mg, 50%; M+H 713.

Step 3: (5R,6Z)-6-{[5-(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)]methylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzy-6-[(acetyloxy)[5(phenylacetyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (300 mg, 0.4 mmol) was dissolved in THF (50 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL). This was hydrogenated at 40 psi pressure, in the presence of 10% Pd/C (80 mg) for 2 hrs. at the end, reaction mixture was filtered through a pad of celite and concentrated. The separated yellow solid was dissolved in ethyl acetate and washed well with water. The organic layer was dried and concentrated. The separated yellow solid was triturated with diethyl ether and filtered. The yellow solid was washed well with diethyl ether and it was found to be 95% pure compound. Yield: 160 mg, 91%; Yellow solid; mp. 166-169^{0}C; (M+H) 439 .

Example 37

Preparation of (5R,6Z)-6-[(7-methylimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Ethyl 7-methylimidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl 7-methylimidazo[2,1-b]-benzthiazole-2-carboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from 6-methyl-2-amino benzothiazole (3.2 g, 20 mmol) and ethyl bromopyruvate (4.0 g, 20.4 mmol), 3.0 g (57% Yield) of ethyl 7-methylimidazo[2,1-b]-benzthiazole-2-carboxylate was isolated as brown solid. (M+H) 261.

Step 2: 2-Formyl-7-methylimidazo[2,1-b]-benzthiazole:

To a stirred solution of Ethyl 7-methylimidazo[2,1-b]-benzthiazole-2-carboxylate (4.0 g, 15.38 mmol) in dry THF at -78^0C, DIBAL (1M. solution in toluene) (16.0 ml, 16 mmol) was added. The reaction mixture was stirred at -78^0C and slowly elevated to room temperature. The reaction mixture was stirred at room temperature for 30 minutes and quenched with saturated NH_4Cl. The reaction mixture was extracted with chloroform and washed well with water. The organic layer was dried over anhydrous $MgSO_4$; filtered and concentrated. The residue was purified bt SiO_2 column chromatography by eluting it with chloroform: metrhanol (20:1). Brown solid; (M+H) 217; Yield: 800 mg (24%)

Step 3: 4-Nitrobenzyl-6-[(acetyloxy) (7-methylimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-7-methylimidazo[2,1-b]-benzthiazole (432 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2.0 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (566 mg, 2.0 mmol) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous

solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 400 mg, 31%; M+H 645.

Step-4: (5*R*),(6*Z*)-6-[(7-methylimidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy)(7-methylimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (350 mg, 0.54 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 110 mg, 55%; as yellow crystals; mp 178^{0}C (Dec); (M+H+Na) 392 .

^{1}H NMR (DMSO-d_6) δ 8.56 (s, 1H), 7.93 (d, 1H), 7.83 (s, 1H), 7.38 (d, 1H), 7.07 (s, 1H), 6.51 (s, 2H), 2.42 (s, 3H).

Step 4: (5*R*),(6*Z*)-6-[(7-methylimidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid: (Procedure B)

4-Nitrobenzyl-6-[(acetyloxy)(7-methylimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (350 mg, 0.54 mmol) was dissolved in THF (40 mL) and 6.5 pH phosphate buffer (40 ml) and hydrogenated over Pd/C (10% , 200 mg) at 40 psi pressure for 3 hrs at room temperature. At the end ,

reaction mixture was filtered through a pad of celite and washed with acetonitrile. The reaction mixture was concentrated to 40 ml and cooled to 0° C and pH was adjusted to 8.5 by adding 1N NaOH. The product was directly loaded over HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions were concentarated and the yellow solid was washed with acetone, filtered and dried. Yield: 110 mg, 55% as yellow solid.

Example 38

Preparation of (5*R*), (6*Z*)-6-(4,5,6,7-tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

Step 1: 5,6,7,8-Tetrahydro-[1,2,4]triazolo[1,5-*a*]pyridin-2-ylamine

The 12.7% solution of HCl in ethanol (5.35 mL) and 10% Pd-C (50% wet) (2.5 g) were added to the mixture of [1,2,4]triazolo[1,5-a]pyridin-2-ylamine (2.5 g) in ethanol (72 mL). The reaction mixture was hydrogenated at 400 KPa of H_2 for 3 days at room temperature. The mixture was filtered and concentrated under reduced pressure. The residue was treated with saturated potassium carbonate solution and extracted with chloroform. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The title compound was obtained as a pale yellow solid (2.31 g, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.88-1.94 (m, 2H), 1.98-2.05 (m, 2H), 2.77 (t, 2H, *J* = 6.2 Hz), 3.95 (t, 2H, *J* = 6.2 Hz), 4.09 (brs, 2H).

Step 2: 4,5,6,7-Tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-carboxylic acid ethyl ester

Ethyl bromopyruvate (10.23 g) was added to the mixture of 5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-*a*]pyridin-2-ylamine (5.8 g) in 1,2-dimethoxyethane (320 mL). The reaction mixture was stirred for 5 hours at room temperature and concentrated to 100 mL under reduced pressure. The precipitate was obtained by an addition of diethyl ether (200 mL), followed by filtration. The precipitate was dissolved in ethanol (175 mL) and stirred for 20 hours at 110 °C in shield tube. The reaction mixture was cooled to room

temperature and concentrated under reduced pressure. The residue was treated with saturated potassium carbonate solution and extracted with chloroform. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - methanol (1/1). The title compound was obtained as a pale yellow solid (7.56 g, 77%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.42 (t, 3H, *J* = 7.1 Hz), 2.14-2.25 (m, 4H), 3.11 (t, 2H, *J* = 6.1 Hz), 4.37 (t, 2H, *J* = 5.7 Hz), 4.41 (q, 2H, *J* = 7.1 Hz), 7.57 (s, 1H).

Step 3: 4,5,6,7-Tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-carbaldehyde

1.01 M Diisobutylalminium hydride in toluene (1.06 mL) was added dropwise to the solution of 4,5,6,7-tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-carboxylic acid ethyl ester (100 mg) in dry THF (5 mL) at –78 °C under a nitrogen atmosphere. The reaction mixture was stirred for 30 minutes at –78 °C and treated with ethanol (ca. 1 mL). The mixture was warmed to 0 °C and stirred for 1 h at 0 °C. The reaction solution was diluted with ethyl acetate (20 mL), treated with 0.5 mL saturated ammonium chloride solution, and sonicated for ca. 5 minutes (until a precipitate was deposited enough). The mixture was dried (Na_2SO_4) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was crystallized from dichloromethane and diethyl ether to give the title compound (47.4 mg, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.16-2.27 (m, 4H), 3.14 (t, 2H, *J* = 6.1 Hz), 4.39 (t, 2H, *J* = 5.7 Hz), 7.53 (s, 1H), 10.01 (s, 1H).

Step 4: (5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[4,5,6,7-tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*] indene-2-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

4,5,6,7-Tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-carbaldehyde (2.97 g) was added to the dry acetonitrile (110 mL) solution of anhydrous $MgBr_2$ (4.45 g) under a nitrogen atmosphere at room temperature. The dry THF solution (110 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (2.97 g) was added to the reaction mixture, cooled to –20 °C, and triethylamine (6.45 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. After the mixture was stirred for 1.2 h at –20 °C, acetic anhydride (2.9 mL) was added in one portion. The reaction mixture was warmed to 0

°C and stirred for 16.5 h at 0 °C. The mixture was diluted with ethyl acetate and washed with H_2O and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with ethyl acetate – *n*-hexane (3/1) and then with ethyl acetate – methanol (5/1). The title compound was obtained as a brown amorphous solid (651.6 mg, 13%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.10-2.24 (m, 4H), 2.29 (s, 3H), 3.04-3.07 (m, 2H), 4.28-4.32 (m, 2H), 5.27 (d, 1H, *J* = 13.7 Hz), 5.43 (d, 1H, *J* = 13.7 Hz), 6.19 (s, 1H), 6.91 (s, 1H), 7.01 (s, 1H), 7.49 (s, 1H), 7.59-7.62 (m, 2H), 8.23-8.25 (m, 2H).

Step 5: (5*R*), (6*Z*)-6-(4,5,6,7-tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)-6-{(*RS*)-Acetoxy-[4,5,6,7-tetrahydro-1,3a,3b,8-tetraaza-cyclopenta[*a*]indene-2-yl]-methyl}-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (643.6 mg) was dissolved in THF (9 mL) and acetonitrile (4.2 mL). Freshly activated Zn dust (2.57 g) and 0.5 *M* phosphate buffer (pH 6.4, 13.2 mL) were added to the reaction mixture. The reaction vessel was covered with foil to exclude light. The mixture was vigorously stirred for 2 h at room temperature. The mixture was cooled to 9 °C, and 1 *M* NaOH aqueous solution was added to adjust pH to 7.5. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water. The aqueous layer was concentrated to 20 mL under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (60 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 2.5-10% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (68 mg, 18%, pH 7.4). Mp 175 °C (dec); ^{1}H-NMR (400 MHz, D_2O) δ 1.85-2.03 (m, 4H), 2.85-2.99 (m, 2H), 4.07-4.14 (m, 2H), 6.34 (s, 1H), 6.74 (s, 1H), 6.76 (s, 1H), 7.28 (s, 1H).

Example 39

Preparation of (5R,6E)-6-[(10-benzyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid

Step 1: Preparation of 8-(hydroxymethyl)dibenzo[b,f][1,4]oxazepin-11(10H)-one;

Lithium aluminum hydride (11 mL, 11 mmole) was slowly added to the solution of 11-Oxo-10,11-dihydro-dibenzo[*b,f*][1,4]oxazepine-8-carboxylic acid methyl ester (1.346 g, 5 mmole) in THF under N_2 at room temperature. The reaction mixture was stirred for 1hour and 45 minutes then quenched with 2N of HCl until the pH value reaches 2-3. Removed all the THF by rotary evaporation, and extracted the reaction mixture with ethyl acetate for five times, dried the organic layer with sodium sulfate and filtered and concentrated. Obtained the desired compound (white solid) in 46% yield.

Step 2: Preparation of 11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepine-8-carbaldehyde:

8-(hydroxymethyl)dibenzo[b,f][1,4]oxazepin-11(10H)-one (0.241 g, 1 mmole) in acetonitrile was added to the molecular sieves (1 g) under N_2 at room temperature then 4-methylmorpholine N-oxide (0.175 g, 1.5 mmole) was also added into the reaction mixture. After stirring the mixture for 10 minutes, tetrapropylammonium perruthenate (0.0176 g, 0.05 mmole) was added and the reaction followed by t.l.c. until complete. Dilute the reaction mixture with 10ml of ethyl acetate and flashed it through a small silica gel column. Collected all the ethyl acetate that contains desired material, extracted the organic layer with 1N HCl and also washed it with brine. Dried the organic layer over sodium sulfate and filtered and concentrated. Obtained the desired compound (white solid) in 83% yield.

Step 3: Preparation of 10-benzyl-11-oxo-10,11-dihydro-dibenzo[b,f][1,4]oxazepine-8-carbaldehyde:

Potassium carbonate anhydrous (0.207g, 1.5 mmole) and benzyl bromide (0.205 g, 1.2

mmole) were added to a solution of the 11-oxo-10,11dihydrodibenzo[b,f][1,4]oxazepine-8-carbaldehyde (0.240 g, 1 mmole) in acetonitrile under N_2 at room temperature. The reaction mixture then was refluxed for 4 hours, and cooled to room temperature. Diluted the reaction mixture with ethyl acetate and filtered through a magnesol pad and concentrated. Purified with silica gel column and 50% ethyl acetate in hexane. Obtained the desired compound (light yellow oil) in 63% yield.

Step 4: Preparation of 6-[acetoxy-(10-benzyl-11-oxo-10,11-dihydro-dibenzo[b,f][1,4]oxazepin-8-yl)-methyl]-6-bromo-7oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester:

10-benzyl-11-oxo-10,11-dihydro-dibenzo[b,f][1,4]oxazepine-8-carbaldehyde (0.250 g, 0.759 mmole) in acetonitrile was added to magnesium bromide (0.419 g, 2.28mmole) under N_2 at room temperature. The dry THF solution of (5R,6S)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (0.292 g, 0.758 mmole) then was added to the mixture. After 15 minutes the reaction mixture was cooled to -20^0C, and triethylamine (0.317 mL, 2.27 mmole) was added. The reaction flask was covered with foil to exclude light. After 4 hours at -20^0C, treated with acetic anhydride (0.358 mL, 3.795 mmole) and DMAP (0.00927 g, 0.0759 mmole). Warmed up the reaction mixture to 0^0C and placed it in freezer overnight. Reaction solution was concentrated and dissolved with ethyl acetate and washed with 5% of citric acid aqueous solution, saturated $NaHCO_3$, water and brine. Organic layer was dried in sodium sulfate and filtered and concentrated. Purified with silica gel column and 1:15 ethyl acetate/CH_2Cl_2. Obtained the desired compound (light yellow oil) in 41% yield.

Step 5: Preparation of 6-(10-benzyl-11-oxo-10,11-dihydro-dibenzo[b,f][1,4]oxazepin-8-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt;

A 0.5M phosphate buffer solution (pH 6.5) was added to a solution of 6-[acetoxy-(10-benzyl-11-oxo-10,11-dihydro-dibenzo[b,f][1,4]oxazepin-8-yl)-methyl]-6-bromo-7oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (0.210 g, 0.273 mmole) in THF, followed by 10% Pd-C (0.0546 g). The reaction mixture then was hydrogenated at 40psi for three hours. Filtered through a celite pad and removed the THF by rotary evaporation, extracted the mixture with ethyl acetate and washed with

water and brine. Dried the organic layer with sodium sulfate and filtered and concentrated. Dissolved the $NaHCO_3$ with minimal amount of distal water and added it to the reaction mixture along with a small amount of ethyl acetate until the pH value reaches 7-8, evaporated the ethyl acetate. Purified with reverse phase column (MCI Gel CHP20P) with varying amounts of acetonitrile (0%-20%) in water. Removed the acetonitrile and water by rotary evaporation, and freeze-dried the compound. Obtained the desired material (yellow solid) in 24% yield.Mp: 179°C. ^{1}H NMR (DMSO) δ 1.755-1.825 (s, 1H), 2.497-2.506 (d, 2H), 5.243-5.434 (m, 2H), 6.516-6.770 (m, 1H), 7.039-7.792 (m, 11H).

Example 40

Preparation of 6-(5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 4-ethoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine
(SM:Ross, L. O.; Goodman, L.; Baker, B. R. J. Am. Chem. Soc. 1959, 81, 3108)

5.1 grams of 4-chloro-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine was dissolved in 200ml xylene and 30 ml absolute ethanol. Then 6.8 gram for sodium ethoxide was added and the mixture was refluxed for 3 hours. Then the solvent was removed in vacuo and 100ml water was added to the residue. Filter and wash the cake with water (50ml). The solid was further vacuumed to dry for several hours. The desired product weighed 5.3 gram (98% yield). Mp: 133.8~134.9 oC.
H-NMR: (300 MHz, $CDCl_3$) δ. 6.23(s, NH2), 4.28(quartet, 2H, J= 6.9 Hz), 2.6 (m, 2H), 1.93 (m, 2H), 1.27 (t, CH3, J=6.9 Hz); MS: 180.0 (M+H)

Step 2: Preparation of 5-Ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester:
5.2 gram (29mmol) 4-ethoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine was dissolved in 100 ml dry THF. Bromopyruvate (5.4ml,) was then added dropwise with in five minutes. The mixture was stirred at 23oC for one hour. It was then filtered and washed with ether to give 8.7 gram of solid. This solid was then dissolved in 50ml

ethanol and refluxed for two hours. The reaction mixture was cooled to room temperature and partitioned between 350ml chloroform and 200 ml saturated sodium bicarbonate. The organic layer was separated and dried over magnesium sulfate. Filter off the drying agent and concentrate to give 6.5 gram of product.

MP: 168.6~168.7 oC.

H-NMR: (300 MHz, $CDCl_3$) δ. 7.69(s, 1H), 4.50 (qartet, 2H, J=7.2 Hz), 4.40 (qartet, 2H, J=7.2 Hz), 3.11 (t, 2H, J=9.6 Hz), 2.88 (t, 2H, J=9.6 Hz), 2.88 (m, 2H), 1.43 (t, 2H, J=7.2 Hz).; MS: 276.2(M+H)

Step 3: Preparation of 5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carbaldehyde

1.925 grams 5-ethoxy-7, 8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester was dissolved in 40 ml dichloromethane and then cooled to –78oC. DIBAL (1 M, 21 ml, 3 eq.) was then added within five minutes. The reaction media was then quenched with 2ml ethanol and partitioned between 350ml dichloromethane and 100 ml 1 N sodium hydroxide. The aqueous layer was washed with another 150ml chloroform and the combined organic layer was dried over magnesium sulfate and filtered and concentrated to give the corresponding alcohol. The alcohol is then dissolved in 150ml dichloromethane and 10 grams of manganese dioxide is then added. The mixture was stirred at 23 oC for two hours. The reaction mixture was then filtered through a pad of celite and concentrated to give 1.1 gram (68%) of the desired aldehyde.

MP: 237.2~237.3° C

H-NMR: (300 MHz, $CDCl_3$) δ. 9.94(s, 1H, CHO), 8.39 (s, 1H), 4.46 (quartet, 2H, J= 7.2Hz), 3.2 (m, 2H, CH2), 2.85 (m, 2H, CH2), 2.24 (m, 2H, CH2), 1.38 (t, 3H, CH3, J=7.2Hz); MS: 232.1(M+H)

Step 4: Preparation of 6-[acetoxy-(5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester:

A 30 ml acetonitrile solution of 5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carbaldehyde (693 mg, 3mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

(1.l55 gram, 1 eq.) was injected within a minute and the reaction mixture was then cooled to –20oC. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml, eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 1.1gram product.

MP: 118.7~119.1 ^{0}C

H-NMR: (300 MHz, $CDCl_3$) δ. 8.35(d, 2H, J=11Hz), 7.63 (m, 2H), 7.41 (d, 1H, J=6.9Hz), 7.08 (d, 1H, J=11Hz), 6.47(s, 1H), 5.55 (4H, CH2), 4.54 (m, 2H), 3.09 (m, 2H), 2.93 (m, 2H), 2.32 (m, 2H), 1.41 (t, J=9.6Hz); MS: 660.1(M+H)

Step 5: Preparation of 6-(5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

6-[acetoxy-(5-ethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.03 gram, 1.565 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 100 mg of product was collected.

MP: >250^0 C

H-NMR: (300 MHz, $CDCl_3$) δ. 7.52 (s, 1H), 6.95(s, 1H), 6.54(s, 1H), 4.73 (m, 2H), 3.06(m, 2H), 2.84 (m, 2H), 2.27 (m, 2H), 1.43 (t, 3H); MS: 383.2 (M+H).

Example 41

(5R,6E&Z)-7-oxo-6-(4H,10H-pyrazolo[5,1-c][1,4]benzoxazepin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 1-(2-fluorobenzyl)-1H-pyrazole-3,5-dicarboxylate

2-fluorobenzyl bromide (2.0 ml, 16.58 mmol) was added to a mixture of diethyl 3,5-pyrazoledicarboxylate (3.01 g, 14.18 mmol), Cs_2CO_3 (5.57 g, 17.1 mmol), and acetonitrile (140 ml) under N_2. Heated to 60°C for two hours and then cooled to room temperature. Filtered and concentrated the reaction solution. Added water (~200mL) to the resulting residue and extracted with EtOAc. Washed organics with water and brine. Dried organics over sodium sulfate and filtered and concentrated. Obtained diethyl 1-(2-fluorobenzyl)-1H-pyrazole-3,5-dicarboxylate (light-yellow oil) in quantitative yield.

Step 2: Preparation of 1-(2-fluorobenzyl)-1H-pyrazole-3,5-methanediol

A 1M solution of DIBAL-H in THF (90 ml, 90 mmol) was added to a solution of diethyl 1-(2-fluorobenzyl)-1H-pyrazole-3,5-dicarboxylate (4.80 g, 14.99 mmol) in CH_2Cl_2 (90 ml) at 0°C under N_2. After two hours quenched with $NH_4Cl_{(aq)}$ and suspension was formed. Filtered and extracted with EtOAc and washed with brine. Dried organics over sodium sulfate and filtered and concentrated. Purified with silica gel column and 5% MeOH in CH_2Cl_2. Obtained 3.4 g of the diol compound (clear oil) in 96% yield.

Step 3: Preparation of 4H,10H-pyrazolo[5,1-c][1,4]benzoxazepine-2-carbaldehyde

The diol compound (3.83 g, 16.21 mmol) in HMPA (24 ml) was added to a suspension of NaH (60%, 1.34 g, 33.5 mmol) in toluene (330 ml) under N_2. Rapidly heated to 95°C for three hours and cooled to room temperature. Quenched with water and extracted with EtOAc. Washed organics with water and brine. Dried organics over sodium sulfate and filtered and concentrated. Purified with silica gel column and 2% MeOH in CH_2Cl_2. Obtained 4H,10H-pyrazolo[5,1-c][1,4]benzoxazepin-2-ylmethanol (white solid). Yield: 0.71 g 20%.

4H,10H-pyrazolo[5,1-c][1,4]benzoxazepin-2-ylmethanol (0.71 g, 3.28 mmol), 4-methylmorpholine N-oxide (1/198g, 10.23 mmol), molecular sieves (powder, 4 angstroms) (3.32 g), and acetonitrile (0.07M) were placed together under N_2. Tetrapropylammoniumperruthenate (0.113 g, 0.322 mmol) was added and after three hours the reaction solution was filtered through celite and concentrated. Purified with silica gel column and 1:1 EtOAc/Hexane. Obtained 4H,10H-pyrazolo[5,1-c][1,4]benzoxazepine-2-carbaldehyde (white solid). Yield: 0.31 g 44%.

Step 4: Preparation of Preparation of 6-[acetoxy-(4H,10H-pyrazolo[5,1-c][1,4]benzoxazepine-8-yl)-methyl]-6-bromo-7oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester:

4H,10H-pyrazolo[5,1-c][1,4]benzoxazepine-2-carbaldehyde (0.19 g, 0.887 mmol) in acetonitrile (14 ml) was added to $MgBr_2$ (0.49g, 2.66 mmol) under N_2. After 25 minutes 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (0.342g, 0.888 mmol.) in THF (14 ml) was added. After 15 minutes the reaction was cooled to -20^0C. Ten minutes later added Et_3N (3eq) and placed reaction in the dark. After 6.5 hours added Ac_2O (0.42 ml, 4.45 mmol) and DMAP (0.011g, 0.0900 mmol). Warmed to 0^0C and placed in freezer overnight. Reaction solution was concentrated and resulting residue was taken up in EtOAc. Washed with 5% citric $acid_{(aq)}$ and saturated $NaHCO_{3(aq)}$. Further washed with water and brine. Dried organics over sodium sulfate and filtered and concentrated. Purified with silica gel prep plates and 1:2 EtOAc/Hexane. Obtained the condensation product (yellow gum/solid). Yield: 0.31 g, 54% yield.

Step 5: (5R,6E&Z)-7-oxo-6-(4H,10H-pyrazolo[5,1-c][1,4]benzoxazepin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt:

Step 6: A 0.5M phosphate buffer solution (pH 6.5) (18mL) was added to a solution of the condensation product (5) (0.300g, 0.468mmol) in THF (18mL). The Pd on Carbon (0.102g) was added and the reaction mixture was hydrogenated at 40psi for two hours. Filtered through celite and removed THF by rotary evaporation. Extracted with EtOAc. Dried organics over sodium sulfate and filtered and concentrated. $NaHCO_3$ (0.08g, 0.952mmol) was dissolved in a minimal amount of water and added to the concentrated organics along with a small amount of EtOAc. Filtered and removed EtOAc by rotary evaporation. Purified with reverse phase column (MCI Gel CHP20P) and varying amounts of acetonitrile (0% to 15%) in water. Removed the acetonitrile and most of the water from the collected fractions by rotary evaporation. Freeze-dried the rest to obtain 41mg of (5R,6E)-7-oxo-6-(4H,10H-pyrazolo[5,1-c][1,4]benzoxazepin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt (6) (yellow solid) in 22% yield. HPLC found the purity to be 77% and the E/Z isomer ratio to be 3:2. ^{1}H-NMR (δ, DMSO-d_6) 5.366 (m, 4H), 5.649 (m, 4H), 6.326 (t, 2H), 6.444 (s, 2H), 6.551 (s, 2H),

6.640 (s, 2H), 6.810 (s, 2H), 6.974 (m, 2H), 7.249 (m, 2H), 7.355 (m, 2H). m/z (M+H)390.0

Example 42

(5*R*), (6*Z*)-6-(5*H*-Imidazo[2,1-*a*]isoindol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

Step 1: Preparation of 5*H*-Imidazo[2,1-*a*]isoindole-2-carbaldehyde

The solution of 2-bromo-3-isopropoxy-propenal (4.97 g) in dry acetonitrile (3 mL) was added to the mixture of 3-amino-1*H*-isoindole (3.4 g) in dry acetonitrile (100 mL). The reaction mixture was stirred for 3.25 h at room temperature. Then triethylamine (3.6 mL) was added to the mixture and heated to reflux for 2 h. The mixture was cooled to room temperature, diluted with ethyl acetate, and washed with 20% potassium hydrogen carbonate. After filtration through a pad of Celite, the organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (3/1 ~ 4/1). The crude compound was crystallized from ethyl acetate and *n*-hexane to give the title compound (1.04 g, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.01 (s, 2H), 7.28-7.52 (m, 3H), 7.90 (s, 1H), 7.91-7.93 (m, 1H), 9.92 (s, 1H).

Step 2: Preparation of (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5*H*-imidazo[2,1-*a*]isoindol-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester:

5*H*-Imidazo[2,1-*a*]isoindole-2-carbaldehyde (736.8 mg) was added to the dry acetonitrile (50 mL) solution of anhydrous $MgBr_2$ (1.8 g) under a nitrogen atmosphere at room temperature. The dry THF solution (50 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.55 g) was added to the reaction mixture, cooled to –20 °C, and triethylamine (1.34 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (0.76 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 18 h at 0 °C. The mixture was diluted with ethyl acetate and washed with H_2O, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite.

The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then eluted with ethyl acetate - hexane (2/3 ~ 1/1). The title compound was obtained as two diastereo mixture (5/1, a pale yellow amorphous solid, 1.8 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 0.84 x 3H), 2.27 (s, 0.16 x 3H), 4.89-4.94 (m, 2H), 5.29 (d, 1H, *J* = 13.6 Hz), 5.47 (d, 1H, *J* = 13.6 Hz), 6.18 (s, 0.16 x 1H), 6.40 (s, 0.84 x 1H), 6.42 (s, 0.84 x 1H), 6.94 (d, 0.16 x 1H, *J* = 0.9 Hz), 7.18 (d, 0.16 x 1H, *J* = 0.7 Hz), 7.35-7.48 (m, 3H), 7.51 (s, 0.84 x 1H), 7.60-7.64 (m, 2H), 7.79-7.83 (m, 1H), 8.23-8.27 (m, 2H).

Step 3: (5*R*), (6*Z*)-6-(5*H*-Imidazo[2,1-*a*]isoindol-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5*H*-imidazo[2,1-*a*]isoindol-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.5 g) was dissolved in THF (21 mL) and acetonitrile (9.8 mL). Freshly activated Zn dust (6 g) and 0.5 *M* phosphate buffer (pH 6.4, 30.8 mL) were added to the reaction mixture. The reaction vessel was covered with foil to exclude light. The mixture was vigorously stirred for 2 h at room temperature. The mixture was cooled to 9 °C, and 1 *M* NaOH aqueous solution was added to adjust pH to 7.5. The reaction solution was mixed with ethyl acetate and filtered through a pad of Celite. The pad was washed with water and the aqueous layer was separated. The aqueous layer was concentrated to 25 mL under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (100 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with water and then with 5-15% acetonitrile-water. The combined fractions was concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (527 mg, 58%). Mp 170 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 4.62 (s, 2H), 6.27 (s, 1H), 6.56 (s, 1H), 6.78 (s, 1H), 7.22-7.31 (m, 4H), 7.52 (d, 1H, *J* = 6.7 Hz).

Example 43

Preparation of (5*R*),(6*Z*)-6-(5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazine-2-carbald hyde

m-Chloroperbenzoic acid (cont. 69%) (6.36 g) was added to the CH_2Cl_2 (111 mL) solution of 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (1.86 g) at 0 °C. The reaction mixture was stirred for 0.5 h at the same temperature and stirred for 18 h at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was triturated with 10 mL of THF and filtered to obtain crystals. The filtrate was concentrated under reduced pressure. The residue was triturated with 5 mL of THF and filtered to obtain crystals. The combined crystals were dried under reduced pressure to give the titled compound as colorless crystals (1.96 g, 89%).

^{1}H NMR ($CDCl_3$) δ 3.60 (t, 2H, *J* = 6.1 Hz), 4.47 (s, 2H), 4.87 (t, 2H, *J* = 6.1 Hz), 6.71 (s, 1H), 9.94 (s, 1H).

Step 2: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5,5-dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester

5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazine-2-carbaldehyde (1.95 g) was added to the dry acetonitrile (112 mL) solution of anhydrous $MgBr_2$ (cont. 98%) (5.48 g) under a nitrogen atmosphere at room temperature. The dry THF solution (112 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.88 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (3.79 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with acetic anhydride (cont. 97%) (3.79 mL) and DMAP (cont. 99%) (120 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. To the reaction mixture was added acetic anhydride (cont. 97%) (0.95 mL) and DMAP (cont. 99%) (120 mg) in one portion. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$), followed by concentration under reduced pressure. The residue was purified with a silica-gel column chromatography ($CHCl_3$: acetone = 19 :1 – 4 : 1) to give the titled compound as a pale brown amorphous solid (diastereo-mixture (8 : 2), 1.35 g, 22%).

^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H x 0.2), 2.25 (s, 3H x 0.8), 3.45 – 3.60 (m, 2H),

4.39 (d, 1H, *J* = 17.0 Hz), 4.44 (d, 1H, *J* = 17.0 Hz), 4.65 – 4.78 (m, 2H), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 1H x 0.8, *J* = 13.5 Hz), 5.44 (d, 1H x 0.2, *J* = 13.5 Hz), 6.05 (s, 1H x 0.8), 6.20 (s, 1H x 0.8), 6.22 (s, 1H x 0.2H), 6.38 (s, 1H x 0.2), 6.39 (s, 1H x 0.2), 6.79 (s, 1H x 0.8), 7.42 (s, 1H x 0.8), 7.44 (s, 1H x 0.2), 7.60 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz).

Step 3: (5*R*),(6*Z*)-6-(5,5-Dioxo-4,5,6,7-tetrahydro-5λ^6-pyrazolo[5,1-*c*][1,4]thiazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy-(5,5-dioxo-4,5,6,7-tetrahydro-5□^{6}-pyrazolo[5,1-*c*][1,4]-thiazin-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (1.33 g) was dissolved in THF (19 mL) and acetonitrile (9 mL). Freshly activated Zn dust (5.32 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 27 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The insoluble material was filtered off and was washed with H_2O (27 mL). The filtrate was added H_2O (27 mL) and washed with ethyl acetate (27 mL) and the aqueous layer was cooled to 3 °C and 1 *M* HCl was added to adjust pH to 2.5. The mixture was stirred for 1 d at the same temperature and added H_2O (55 mL), then stirred for 4 d at the same temperature. The mixture was stirred for 10 h at room temperature. The resultant mixture was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8. The mixture was concentrated under high vacuum at 35 °C. The concentrate was treated to Diaion HP-21 (80 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 9/1). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (306 mg, 38%, pH 7.4).

Mp 180 °C (dec); ^{1}H NMR (D_2O) δ 3.83 (t, 2H, *J* = 6.1 Hz), 4.68 (s, 2H), 4.72 (t, 2H, *J* = 6.1 Hz), 6.37 (s, 1H), 6.40 (s, 1H), 6.95 (s, 1H), 6.98 (s, 1H).

Example 44

Preparation of (5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Piperazine-2-carboxylic acid, dihydrochloride:

The titled compound was prepared in the same way of M. T. Wu and co-workers (*Bioorg. Med. Chem. Lett.* **1993**, *3*, 2023-2028).

Step 1: Piperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester

$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (15.8 g) was added to the H_2O (275 mL) solution of piperazine-2-carboxylic acid, dihydrochloride (22.3 g), then the mixture was refluxed and stirred for 10 min. The insoluble material was filtered off and was washed with hot H_2O (165 mL). The filtrate was cooled to room temperature, and $NaHCO_3$ (9.2 g) and 1,4-dioxane (220 mL) was added to the dark blue solution. The mixture was cooled to 0 °C and $NaHCO_3$ (18.5 g) and 50% solution of 4-nitrobenzyl chloroformate in 1,4-dioxane (61.7 g) was added to the mixture for 0.5 h. After stirring for additional 1.5 h at 0 °C, the precipitate was filtered and washed with cold H_2O (140 mL), EtOH (100 mL), acetone (200 mL) and Et_2O (100 mL), then it was allowed to dry under reduced pressure to obtain the pale blue crystals. The crystals were added to the 1 mol/L HCl (330 mL) solution of EDTA·2Na (20.5 g) for 30 min, and stirred for 2 h at room temperature. The suspension was filtered and the filtered material was diluted with EtOH – H_2O (7 : 3, 550 mL) and refluxed for 10 min. The reaction mixture was filtered to obtain the colorless crystals. The recrystallization from the filtrate was carried out 3 times to obtain additional crystals. The combined crystals were dried under reduced pressure to obtain the titled compound (26.25 g, 77%) as colorless crystals.

^{1}H NMR (D_2O) δ 2.54 – 2.61 (m, 1H), 2.89 (dt, 2H, J = 12.7, 3.4 Hz), 2.97 (br, 1H), 3.13 (br, 1H), 3.62 – 4.04 (m, 2H), 5.16 (s, 2H), 7.49 (d, 2H, J = 8.6 Hz), 8.14 (d, 2H, J = 8.6 Hz).

Step 2: 5-(4-Nitrobenzyloxycarbonyl)-3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-1,5-diaza-7a-azoniainden-3a-ide

The H_2O (300 mL) solution of $NaNO_2$ (cont. 98.5 %) (6.66 g) was added to the acetic acid (864 mL) solution of piperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester (26.72 g) under a nitrogen atmosphere at 0 °C for 0.5 h and stirred for 1 h. In addition, the H_2O (132 mL) solution of $NaNO_2$ (cont. 98.5 %) (2.41 g) was added to the solution at 0 °C for 0.5 h and stirred for 1 h. The solution was concentrated under reduced pressure and H_2O (500 mL) was added to the residue. The solution was extracted with AcOEt (5 times) and organic layer was washed with brine. The mixture was dried over

$MgSO_4$, filtered and concentrated under reduced pressure to afford crude 4-nitrosopiperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester as pale brown amorphous (27.83 g (gross), 25.77 g (net), 88.2%).

The THF (10 mL) solution of trifluoroacetic anhydride (24.0 g) was added to the THF (371 mL) solution of crude 4-nitrosopiperazine-1,3-dicarboxylic acid 1-(4-nitrobenzyl) ester under a nitrogen atmosphere at 0 °C for 15 min. The solution was stirred for 1.5 h at 0 °C and for 1h at room temperature. The THF (5 mL) solution of trifluoroacetic anhydride (8.0 g) was added to the solution for 5 min and stirred for 20 h at room temperature. To the solution was added trifluoroacetic anhydride (8.0 g) for 5 min and the solution was stirred for 4h. The precipitate was filtered and washed with THF and Et_2O. The filtrate was concentrated under reduced pressure. The residue was triturated with THF, filtered and washed with Et_2O. These materials were combined and dried under reduced pressure to afford the titled compound as colorless crystals (22.3 g, 91%).

^{1}H NMR ($CDCl_3$) δ 4.06 (t, 2H, *J* = 5.4 Hz), 4.37 (t, 2H, *J* = 5.4 Hz), 4.63 (s, 2H), 5.30 (s, 2H), 7.54 (d, 2H, *J* = 8.7 Hz), 8.25 (d, 2H, *J* = 8.7 Hz).

Step 3: 6,7-Dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-2,5-dicarboxylic acid 2-ethyl ester 5-(4-nitrobenzyl) ester

Ethyl propiolate (cont. 99%)(8.28 g) was added to the *o*-xylene (348 mL) solution of 5-(4-nitrobenzyloxycarbonyl)-3-oxo-3a,4,6,7-tetrahydro-3*H*-2-oxa-1,5-diaza-7a-azoniainden-3a-ide (22.3 g) under a nitrogen atmosphere and refluxed for 16 h. The solution was concentrated under reduced pressure, followed by silica-gel column chromatography 3 times (*n*-hexane /AcOEt = 2/1 – 1/3). The titled compound was obtained as pale yellow crystals (16.78 g, 64%). Besides, 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-3,5-dicarboxylic acid 3-ethyl ester 5-(4-nitrobenzyl) ester was obtained as pale yellow crystals (6.18 g, 24%).

^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 4.01 (t, 2H, *J* = 5.5 Hz), 4.31 (t, 2H, *J* = 5.5 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 4.79 (s, 2H), 5.29 (s, 2H), 6.64 (s, 1H), 7.54 (d, 2H, *J* = 8.6 Hz), 8.24 (d, 2H, *J* = 8.6 Hz).

Step 4: 2-Hydroxymethyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

$LiBH_4$ (640 mg) and MeOH (1.2mL) was added to the THF (267 mL) solution of 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-2,5-dicarboxylic acid 2-ethyl ester 5-(4-nitrobenzyl) ester (10 g) under a nitrogen atmosphere at room temperature and stirred for 3 h at 40 °C. Additional $LiBH_4$ (523 mg) and MeOH (1.0 mL) was added to the solution and stirred for 1 h at 40 °C and 1 h at 50 °C. The mixture was acidified with 3 mol/L HCl to pH 2 and stirred for 1 h at room temperature, then solid K_2CO_3 was added to the solution to adjust pH to 8. The insoluble material was filtered off and the filtrate was extracted with AcOEt. The organic layer was dried (K_2CO_3), and concentrated under reduced pressure. The residue was purified with silica gel column chromatography ($CHCl_3$ / MeOH =49/1 - 19/1) to afford titled compound as pale yellow crystals (8.44 g, 95%).

^{1}H NMR ($CDCl_3$) δ 1.69 (br, 1H), 3.98 (t, 2H, *J* = 5.5 Hz), 4.19 (t, 2H, *J* = 5.5 Hz), 4.65 (s, 2H), 4.75 (s, 2H), 5.28 (s, 2H), 6.08 (s, 1H), 7.53 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz).

Step 5: 2-Formyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

MnO_2 (activated) (84.2 g) was added to the $CHCl_3$-MeOH (95 : 5, 253 mL) solution of 2-hydroxymethyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester (8.42 g), and the mixture was refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. Silica-gel (20 g) was added to the filtrate and the solvent was removed under reduced pressure to give the silica-gel coating with crude reactant. The above silica-gel was adsorbed to silica-gel column chromatography and the column was eluted with $CHCl_3$ - MeOH (49/1 to 19/1). The titled compound was obtained as yellow crystals (2.82 g, 34%).

^{1}H NMR ($CDCl_3$) □4.05 (t, 2H, *J* = 5.5 Hz), 4.32 (t, 2H, *J* = 5.5 Hz), 4.81 (s, 2H), 5.29 (s, 2H), 6.62 (s, 1H), 7.54 (d, 2H, *J* = 8.7 Hz), 8.24 (d, 2H, *J* = 8.7 Hz), 9.93 (s, 1H).

Step 6: 2-{(*RS*)-Acetoxy-[(5*R*, 6*RS*)-6-bromo-2-(4-nitrobenzyloxycarbonyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl]-methyl}-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester

2-Formyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester (2.71 g) was added to the dry acetonitrile (164 mL) solution of anhydrous $MgBr_2$

(cont. 98%) (6.17 g) under a nitrogen atmosphere at room temperature. The dry THF solution (164mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.27 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (9.24 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 1.5 h at –20 °C and treated with acetic anhydride (cont. 97%) (3.19 mL) and DMAP (cont. 99%) (203 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 1 h at 0 °C. Acetic anhydride (3.19 mL) was added to the solution and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, water and brine. The organic layer was dried ($MgSO_4$), followed by concentration under reduced pressure. The residue was purified with silica-gel column chromatography three times (*n*-hexane – AcOEt (1/1 to 2/3), $CHCl_3$ – acetone (29/1 to 19/1) and $CHCl_3$ – acetone (29/1)). The titled compound was obtained as yellow amorphous (diastereo-mixture (64 : 36), 3.30 g, 53%).

^1H NMR ($CDCl_3$) δ 2.06 (s, 3H x 0.36), 2.26 (s, 3H x 0.64), 3.95 – 4.04 (m, 2H), 4.18 (s, 2H), 4.73 (d, 1H, *J* = 18.2 Hz), 4.78 (d, 1H, *J* = 18.2 Hz), 5.28 (d, 1H, *J* = 13.5 Hz), 5.28 (s, 2H), 5.43 (d, 1H x 0.64, *J* = 13.5 Hz), 5.44 (d, 1H x 0.36), 6.06 (s, 1H x 0.64), 6.08 (s, 1H x 0.64), 6.24 (s, 1H x 0.36), 6.27 (s, 1H x 0.36), 6.41 (s, 1H x 0.36), 6.79 (s, 1H x 0.64), 7.42 (s, 1H x 0.64), 7.44 (s, 1H x 0.36), 7.53 (d, 2H, *J* = 8.6 Hz), 7.60 (d, 2H, *J* = 8.8 Hz), 8.24 (d, 2H, *J* = 8.8 Hz), 8.24 (d, 2H, *J* = 8.6 Hz).

<u>Step 7:</u> (5*R*),(6*Z*)-7-Oxo-6-(4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazin-2-ylmethylene)-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

To the THF (43 mL) and acetonitrile (20 mL) solution of 2-{(*RS*)-acetoxy-[(5*R*,6*RS*)-6-bromo-2-(4-nitrobenzyloxycarbonyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl]-methyl}-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrazine-5-carboxylic acid 4-nitrobenzyl ester was added Zn dust (12.36 g) rapidly with 0.5 *M* phosphate buffer (pH 6.5, 63 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The insoluble material was filtered off and was washed with H_2O (63 mL). The filtrate was washed with ethyl acetate (63 mL) and the aqueous layer was cooled to 3 °C and 1 *M* HCl was added to adjust pH to 2.5. The mixture was stirred for 4 h at the same temperature and added H_2O (63 mL) and 1

M HCl to adjust pH to 2.5, then stirred for 17 h at the same temperature. To the mixture was added 1 *M* NaOH to adjust pH to 8. The mixture was concentrated under high vacuum at 35 °C. The concentrate was treated to Diaion HP-21 (124 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 – 95/5). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the title compound as a yellow amorphous solid (288 mg, 22%, pH 8.8).

Mp 160 °C (dec); ^{1}H NMR (D_2O) δ 2.94(t, 2H, *J* = 5.6 Hz), 3.67 (d, 1H, *J* = 17.2 Hz), 3.70 (d, 1H, *J* = 17.2 Hz), 3.82 (t, 2H, *J* = 5.6 Hz), 5.84 (s, 1H), 6.03 (s, 1H), 6.65 (s, 1H), 6.67 (s, 1H).

Example 45

Preparation of (5*R*)(6*Z*)-6-(5,5-Dimethyl-4*H*-1,6a-diazapentalen-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt 5,5-Dimethyl-2-piperidone

5-5-Dimethyl-2-piperadinone was prepared in the method of Nagasawa (*J. Med. Chem.*, **20**, 1176 (1977)).

Step 1: 3,3-Dichloro-5,5-dimethyl-2-piperidone

To a cold (0 °C) stirred solution of 5,5-dimethyl-2-piperidone (30.2 g, 0.24 mol) in 475 mL of $CHCl_3$, PCl_5 (57.1 g, 0.26 mol) was added at such a rate that the temperature never exceeded 7 °C. After the addition was complete, stirring was continued for 10 min. Sulfuryl chloride (96.6 g, 0.72 mol) was slowly added and the mixture was heated under reflux for 1 h. The solution was concentrated under reduced pressure. The residue was cooled in ice and diluted with 250 mL of ice-water. The product was then extracted with $CHCl_3$ (6 x 250 mL) and the organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with $CHCl_3$–MeOH (50 : 1). The titled compound was obtained as a white solid (41.3 g, 88.8 %). (*J. Med. Chem.*, **20**, 1176 (1977))

^{1}H NMR ($CDCl_3$) δ 1.17 (s, 6H), 2.76 (s, 2H), 3.19 (d, 2H, *J* = 3.0 Hz), 6.82 (brs, 1H).

Step 2: 3-Chloro-5,5-dimethyl-2-piperidone

To 40.8 g (0.21 mol) of 3,3-dichloro-5,5-dimethyl-2-piperidone dissolved in 410 mL of AcOH was added 10% Pd/C (50% wet, 6.2 g) and NaOAc-$3H_2O$ (62.4g, 0.46 mol) and the mixture was hydrogenated at 300 kPa for 20 min. The pressure of hydrogen was adjusted at 300 kPa every 5 min. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure. $CHCl_3$ (400 mL) and water (300 mL) were added to the residue and the aqueous layer was neutralized with 4 mol/L NaOH. The mixture was separated and the aqueous layer was extracted with $CHCl_3$ (5 x 300 mL) and the organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with hexane–AcOEt (1 : 1). The titled compound was obtained as a white solid (20.4 g, 59.9 %). (*J. Med. Chem.*, **20**, 1176 (1977))

^{1}H NMR ($CDCl_3$) δ 1.10 (s, 3H), 1.12 (s, 3H), 2.02 (dd, 1H, J = 10.8, 13.6 Hz), 2.20 (ddd, 1H, J = 2.2, 6.7, 13.6 Hz), 2.97 (ddd, 1H, J = 2.3, 3.9, 12.1 Hz), 3.22 (d, 1H, J = 12.1 Hz), 4.44 (dd, 1H, J = 6.8, 10.7 Hz), 6.66 (brs, 1H).

Step 3: 4,4-Dimethylpyrrolidine-2-carboxylic acid

A suspension of 20.4 g (0.13 mol) of 3-chloro-5,5-dimethyl-2-piperidone and 45.2 g (0.14 mol) of $Ba(OH)_3$-$8H_2O$ in 252 mL of water was heated in a Parr apparatus at 150 °C for 6 h. Then, 18.6 g (0.14 mol) of ammonium sulphate were added. The precipitate was filtered off, and the solution was concentrated under reduced pressure to dryness. Crude 4,4-dimethylpyrrolidine-2-carboxylic acid was obtained as a white solid (37.5 g). (*J. Med. Chem.*, **20**, 1176 (1977), EP 0 447 704 A1, page 17)

^{1}H NMR (D_2O) δ 1.10 (s, 3H), 1.11 (s, 3H), 1.88 (dd, 1H, J = 7.8, 13.2 Hz), 2.21 (dd, 1H, J = 9.2, 13.2 Hz), 3.12 (dd, 2H, J = 11.5, 23.5 Hz), 4.22 (dd, 1H, J = 8.1, 8.9 Hz).

Step 4: 5,5-Dimethyl-3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-1-aza-6a-azonio-3a-pentalenide

To a suspension of 37.5 g of the crude 4,4-dimethylpyrrolidine-2-carboxylic acid in 420 mL of AcOH was added a solution of 13.3 g (0.19 mol) of $NaNO_2$ in 210 mL of water over 15 min at room temperature and stirred for 3 h. The solution was concentrated under reduced pressure. Acetone (250 mL) was added to the residue

and the precipitate was filtered off, and the solution was concentrated under reduced pressure to dryness and crude 4,4-dimethyl-1-nitrosopyrrolidine-2-carboxylic acid was obtained as brown oil.

To a solution of crude 4,4-dimethyl-1-nitrosopyrrolidine-2-carboxylic acid in 252 mL of dry THF was added trifluoroacetic anhydride (81.3 g, 0.39 mol) under a nitrogen atmosphere at 0 °C and stirred for 6 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (2 : 1). The titled compound was obtained as a brown solid (12.0 g, 61.7 %).

^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H), 2.71 (s, 2H), 4.12 (s, 2H).

Step 5: 5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethylester

A solution of 5,5-dimethyl-3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-1-aza-6a-azonio-3a- pentalenide (10.8 g, 0.07 mol) and ethyl propiolate (10.8 mL, 0.11 mol) in *o*-xylene (350 mL) was refluxed under a nitrogen atmosphere for 16 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (3 : 1). The titled compound was obtained as a pale brown solid (4.63 g, 31.7 %), and 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-3-carboxylic acid ethylester was obtained as a yellow solid (4.73 g, 32.4 %).

5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethylester: ^{1}H NMR ($CDCl_3$) δ 1,29 (s, 6H), 1.40 (t, 3H, *J* = 7.1 Hz), 2.71 (s, 2H), 3.93 (s, 2H), 4.39 (q, 2H, *J* = 7.1 Hz), 6.54 (s, 1H).

5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-3-carboxylic acid ethylester :^{1}H NMR ($CDCl_3$) δ 1,32 (s, 6H), 1.33 (t, 3H, *J* = 7.1 Hz), 2.89 (s, 2H), 3.90 (s, 2H), 4.26 (q, 2H, *J* = 7.1 Hz), 7.90 (s, 1H).

Step 6: 5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carbaldehyde

To 4.63 g (22.2 mmol) of 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-carboxylic acid ethylester in 222 mL of dry THF was added $LiAlH_4$ (0.85 g, 22.3 mmol) under a nitrogen atmosphere at 0 °C, and then stirred for 1 h. The mixture was quenched with water (5.0 mL) and the precipitate was filtered through a pad of Celite

and the pad was washed with water (50 mL) and THF (150 mL). The filtrate was concentrated under reduced pressure, and then water (50 mL) was added. The aqueous layer was extracted with $CHCl_3$ (5 x 100 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude 5,5-Dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-2-yl)methanol was obtained as a yellow solid (3.19 g).

To 3.19 g of the crude (5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-2-yl) methanol in 222 mL of $CHCl_3$ was added activated MnO_2 (18.5 g) under a nitrogen atmosphere at room temperature, and then refluxed for 1 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to silica-gel column chromatography, and then the column was eluted with hexane-AcOEt (3 : 1). The titled compound was obtained as a brown solid (2.48 g, 68.0 % from the ester).

^{1}H NMR ($CDCl_3$) δ 1.32 (s, 6H), 2.73 (s, 2H), 3.95 (s, 2H), 6.52 (s, 1H), 9.90 (s, 1H).

<u>Step 7:</u> (5*R*)(6*Z*)-6-(5,5-Dimethyl-4*H*-1,6a-diazapentalen-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (16 mL) solution of 5,5-dimethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*] pyrazole-2-carbaldehyde (2.48 g, 15.1 mmol) was added to the dry acetinitrile (90 mL) solution of $MgBr_2$ (3.07 g, 16.4 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 15 min. The dry THF (106 mL) solution of *p*-nitrobenzyl (5*R*, 6*S*)-6-bromopenem-3-carboxylate (5.30 g, 13.8 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (4.6 mL, 33.0 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (172 mg, 1.4 mmol) and acetic anhydride (2.6 mL, 27.6 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. Ethyl acetate (420 mL) and 1mol/L citric acid aqueous solution (210 mL) was added to the reaction mixture and separated. The organic layer was washed with saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(5,5-dimethyl- 4*H*-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-

carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous.

Freshly activated Zn dust (32.0 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 167 mL) to the THF (114 mL) and acetonitrile (53 mL) solution of crude (5*R*)-6-[acetoxy-(5,5-dimethyl- 4*H*-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction solution was cooled at 0 °C, and then the pH was adjusted to 8.0. Ethyl acetate (85 mL) was added to the mixture and filtered through a pad of Celite. The pad was washed with water (120 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layers were cooled at 0 °C, and then the pH was adjusted to 8.5. The mixture was concentrated to 325 g, and then applied to Diaion HP-21 resin (240 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (480 mL) and then acetonitrile aqueous solution (10%; 480 mL, 20%; 720 mL). The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the titled compound as a yellow amorphous solid (2.00 g, 42.8 %, pH 7.16).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ 1.19 (s, 6H), 2.67 (s, 2H), 3.85 (s, 2H), 6.15 (s, 1H), 6.45 (s, 1H), 6.96 (s, 1H), 7.03 (s, 1H); IR (KBr) 3422, 1752, 1683, 1598, 1557 cm^{-1}; λ^{max} (H_2O) 296, 198 nm.

Example 46

Preparation of (5*R*),(6*Z*)- 6-(5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: 5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carboxylic acid methyl ester

The titled compound was prepared according to the procedure of Tim Johnson and co-workers (*Synlett* **2001**, *5*, 646 – 648).

Step 2: (5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methanol

5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carboxylic acid methyl ester (2.24 g) was added to the THF (59 mL) solution of $LiAlH_4$ (511 mg) under a nitrogen atmosphere at 0 °C and stirred for 1 h at 0 °C. The mixture was quenched with 10 mL of water and

filtered. The filtrate was concentrated under reduced pressure and the obtained aqueous solution was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$ and filtered. The filtrate was concentrated to afford titled compound as yellow oil (1.86 g, quant.).

^{1}H NMR ($CDCl_3$) δ 1.66 (t, 1H, *J* = 5.9 Hz), 2.38 – 2.46 (m, 2H), 2.50 – 2.55 (m, 2H), 2.65 – 2.70 (m, 2H), 4.54 (d, 2H, *J* = 5.9 Hz), 6.15 (s, 1H).

Step 3: 5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-carbaldehyde

Activated MnO_2 (9.3 g) was added to the $CHCl_3$ (135 mL) solution of (5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methanol (1.86 g) and refluxed for 1 h under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (9/1 – 7/1). The titled compound was obtained as yellow crystals (1.51 g, 77%).

^{1}H NMR ($CDCl_3$) δ 2.47 – 2.57 (m, 2H), 2.63 (t, 2H, *J* = 6.8 Hz), 2.78 (t, 2H, *J* = 7.3 Hz), 7.06 (s, 1H), 9.44 (s, 1H).

Step 4: (5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester

The acetonitrile solution (50 mL) of 5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-carbaldehyde (1.33 g) was added to the dry acetonitrile (101 mL) solution of anhydrous $MgBr_2$ (cont. 98%) (5.52 g) under a nitrogen atmosphere at room temperature. The dry THF solution (151 mL) of (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (cont. 96.5%) (3.91 g) was added to the mixture, cooled to –20 °C, and Et_3N (cont. 99%) (8.28 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (cont. 97%) (4.13 mL) and DMAP (cont. 99%) (121 mg) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% aqueous solution of citric acid, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) then filtered. The filtrate was concentrated under reduced pressure. The residue was purified with a silica-gel column chromatography (*n*

- hexane : AcOEt = 4 :1 – 3 : 1) to give the titled compound as a brown amorphous solid (3.34 g, 61%).

^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H), 2.40 – 2.48 (m, 2H), 2.53 (t, 2H, *J* = 7.0 Hz), 2.69 (t, 2H, *J* = 7.0 Hz), 5.28 (d, 1H, *J* = 13.5 Hz), 5.43 (d, 1H, *J* = 13.5 Hz), 6.00 (s, 1H), 6.37 (s, 1H), 6.71 (s, 1H), 7.41 (s, 1H), 7.60 (d, 2H, *J* = 8.1 Hz), 8.24 (d, 2H, *J* = 8.1 Hz).

Step 5: (5*R*),(6*Z*)- 6-(5,6-Dihydro-4*H*-cyclopenta[*b*]furan-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

(5*R*, 6*RS*)-6-[(*RS*)-Acetoxy(5,6-dihydro-4*H*-cyclopenta[*b*]furan-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (3.28 g) was dissolved in THF (46 mL) and acetonitrile (21 mL). Freshly activated Zn dust (13.12 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 67 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.25 h at room temperature. The reaction mixture was filtered through a pad of Celite. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was cooled to 3 °C and 1 *M* NaOH was added to adjust pH to 8.0. The mixture was concentrated under high vacuum at 35 °C. The concentrate was applied to Diaion HP-21 (181 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled crude product (288mg). This was purified by Diaion HP-21 (100 mL, Mitsubishi Kasei Co. Ltd.) resin column chromatography. After adsorbing, the column was eluted with H_2O – MeCN (1/0 - 85/15). The combined fractions were concentrated under high vacuum at 35 °C and lyophilized to give the titled compound as a yellow amorphous solid (185 mg, 10%, pH 7.2).

Mp 170 °C (dec); ^{1}H NMR (D_2O) δ 2.24 – 2.30 (m, 2H), 2.37 (t, 2H, *J* = 6.5 Hz), 2.52 – 2.57 (t, 2H, *J* = 7.1 Hz), 6.32 (s, 1H), 6.55 (s, 1H), 6.73 (s, 1H), 6.86 (s, 1H).

Example 47

Preparation of (5*R*)(6*Z*)-6-(4,5-Dihydro-6-thia-1,7a-diazainden-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: *DL*-Tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride

DL-Tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride was prepared according to the method of Lewis (*J. Med. Chem.*, **21**, 1070 (1978)).

Step 2: 4,5-Dihydro-3a*H*,7*H*-2-oxa-3-oxo-6-thia-1-aza-7a-azonioinden

To a suspension of *DL*-tetrahydro-1,3-thiazine-4-carboxylic acid hydrochloride (48.6 g, 0.26 mol) in 666 mL of AcOH was added to the solution of 27.4 g (0.40 mol) of $NaNO_2$ in 333 mL of water over 16 min at room temperature and stirred for 3 h. The solution was concentrated under reduced pressure. Acetone (300 mL) was added to the residue and the precipitate was filtered off. The filtrate was concentrated under reduced pressure to dryness and crude 3-nitroso[1,3]thiazinane-4-carboxylic acid was obtained as brown amorphous solid.

To a solution of crude 3-nitroso[1,3]thiazinane-4-carboxylic acid in 530 mL of dry THF was added trifluoroacetic anhydride (168.4 g, 0.80 mol) over 60 min under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (1 : 2). The titled compound was obtained as brown powder (28.0 g, 67.0 %).

^{1}H NMR ($CDCl_3$) δ 3.00 (t, 2H, *J* = 5.7 Hz), 3.07 (t, 2H, *J* = 5.7 Hz), 5.16 (s, 2H).

Step 3: 4,5-Dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester

A solution of 4,5-dihydro-3a*H*,7*H*-2-oxa-3-oxo-6-thia-1-aza-7a-azonioinden (28.0 g, 0.18 mol) and ethyl propiolate (27.0 mL, 0.27 mol) in *o*-xylene (590 mL) was refluxed under a nitrogen atmosphere for 16 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with *n*-hexane - AcOEt (3 : 1). The titled compound was obtained as pale brown needles (22.1 g, 58.7 %), and 4,5-dihydro-6-thia- 1,7a-diazaindene-3-carboxylic acid ethylester was obtained as pale brown crystals (12.7 g, 33.9 %).

4,5-Dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 2.98 (t, 2H, *J* = 6.1 Hz), 3.21 (t, 2H, *J* = 6.1 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 5.17 (s, 2H), 6.60 (s, 1H).

4,5-dihydro-6-thia- 1,7a-diazaindene-3-carboxylic acid ethylester :^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.1 Hz), 2.99 (t, 2H, *J* = 6.1 Hz), 3.45 (t, 2H, *J* = 6.1 Hz), 4.28 (q, 2H, *J* = 7.1 Hz), 5.11 (s, 2H), 7.85 (s, 1H).

Step 4: 4,5-Dihydro-6-thia-1,7a-diazaindene-2-carbaldehyde

To a 22.1 gram (0.10 mol) of 4,5-dihydro-6-thia-1,7a-diazaindene-2-carboxylic acid ethylester in 520 mL of dry THF was added $LiAlH_4$ (3.95 g, 0.10 mol) under a nitrogen atmosphere at 0 °C, and then stirred for 45 min. The mixture was quenched with water (20 mL) and the precipitate was filtered through a pad of Celite and the pad was washed with water (100 mL) and THF (250 mL). The filtrate was concentrated under reduced pressure, and then water (300 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (6 x 500 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude was obtained as pale yellow crystals (17.2 g).

To a 17.2 gram of the crude (4,5-dihydro-6-thia-1,7a-diazainden-2-yl) methanol in 520 mL of $CHCl_3$ was added activated MnO_2 (88.0 g) under a nitrogen atmosphere at room temperature, and then refluxed for 2 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to silicagel column chromatography, and then the column was eluted with hexane-AcOEt (2 : 1). The titled compound was obtained as yellow crystals (13.0 g, 74.5 %)

^1H NMR ($CDCl_3$) δ 3.00 (t, 2H, J = 6.0 Hz), 3.23 (t, 2H, J = 6.0 Hz), 5.18 (s, 2H), 6.58 (s, 1H), 9.92 (s, 1H).

Step 5: (5*R*)(6*Z*)-6-(4,5-Dihydro-6-thia-1,7a-diazainden-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (11 mL) solution of 4,5-dihydro-6-thia-1,7a-diazaindene-2-carbaldehyde (1.70 g, 10.1 mmol) was added to the dry acetinitrile (60 mL) solution of $MgBr_2$ (2.03 g, 11.0 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 10 min. The dry THF (71 mL) solution of *p*-nitrobenzyl (5*R*, 6*S*)-6-bromopenem-3-carboxylate (3.55 g, 9.2 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (3.1 mL, 22.2 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (0.11 g, 0.9 mmol) and acetic anhydride (1.8 mL, 18.6 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. Ethyl acetate (280 mL) and 1mol/L citric

acid aqueous solution (140 mL) was added to the reaction mixture and separated. The organic layer was washed with saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(4,5-dihydro- 6-thia-1,7a-diazainden-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (21.4 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 112 mL) to the THF (76 mL) and acetonitrile (36 mL) solution of crude (5*R*)-6-[acetoxy-(4,5-dihydro- 6-thia-1,7a-diazainden-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2- carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction solution was cooled at 0 °C, and then the pH was adjusted to 8.0. Ethyl acetate (56 mL) was added to the mixture and filtered through a pad of Celite. The pad was washed with water (150 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 30 mL). The combined aqueous layers were cooled at 0 °C, and then the pH was adjusted to 8.0. The mixture was concentrated to 236 g, and then applied to Diaion HP-21 resin (480 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (960 mL) and then acetonitrile aqueous solution (5%; 960 mL, 10%; 960 mL, 20%; 960 mL). The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the titled compound as a yellow amorphous solid (1.28 g, 40.5 %, pH 7.45).

Mp 200 °C (dec); ^{1}H NMR (D_2O) δ 2.95 (t, 2H, J = 6.1 Hz), 3.12 (t, 2H, J = 6.1 Hz), 5.08 (s, 2H), 6.23 (s, 1H), 6.46 (s, 1H), 6.97 (s, 1H), 7.01 (s, 1H); IR (KBr) 3382, 1752, 1684, 1597, 1554 cm^{-1}; λ^{max} (H_2O) 366, 292, 197 nm.

Example 48

Preparation of (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 5,5-Dimethyl-2-piperidone

5-5-Dimethyl-2-piperadinone (1) was prepared in the method of Nagasawa (*J.*

Med. Chem., **23**, 1176 (1977)).

Step 2: Preparation of 3,3-Dimethyl-6-methoxy-2,3,4,5-tetrahydropyridine

Trimethyloxonium tetrafluoroborate (97%, 11.9 g, 78 mmol) was added to the dry dichloromethane (156 mL) solution of 5,5-dimethyl-2-piperidone (9.93 g, 78 mmol) at room temperature and stirred for 14 h. The reaction mixture was neutralized with 10% sodium hydrogen carbonate aqueous solution, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 120 mL), then the combined organic layer was washed with 10% sodium hydrogen carbonate aqueous solution and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the titled compound was obtained as pale yellow oil (9.0 g, 82.0 %).

^{1}H NMR ($CDCl_3$) δ 0.92 (s, 6H), 1.49 (t, 2H, J = 7.0 Hz), 2.18 (t, 2H, J = 7.0 Hz), 3.19 (s, 2H), 3.63 (s, 3H).

Step 3: 5,5-Dimethylpiperidine-2-ylideneamine monohydrochloride

The mixture of 3,3-dimethyl-6-methoxy-2,3,4,5-tetrahydropyridine (9.0 g, 64 mmol) and ammonium chloride (3.4 g, 64 mmol) in dry ethanol (160 mL) was heated to reflux for 2 h. The reaction mixture was then concentrated under reduced pressure and the titled compound was obtained as a white solid (9.9 g, 94.6 %).

^{1}H NMR (DMSO-d^6) δ 0.95 (s, 6H), 1.52 (t, 2H, J = 6.9 Hz), 2.55 (t, 2H, J = 6.9 Hz), 2.99 (d, 2H, J = 2.1 Hz).

Step 4: 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde & 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde

The mixture of 2-bromo-3-hydroxypropenal (10.1 g, 67 mmol), p-toluenesulfonic acid monohydrate (0.13 g, 0.6 mmol) and 2-propanol (12.6 mL, 165 mmol) in cyclohexane (100 mL) was azeotroped until the vaper temperature over 80°C. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in dry EtOH (200 mL). The dry EtOH (350 mL) solution of 5,5-dimethylpiperidine-2-ylideneamine monohydrochloride (9.9 g, 61 mmol) and the dry EtOH (50 mL) solution of NaOMe (28%, 11.7 g, 61 mmol) were added at room temperature. The reaction mixture was stirred at room temperature for 2 h, and then the reaction solution was removed in

vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and triethylamine (8.5 mL, 61 mmol) was added, and then the reaction mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature, and then the reaction solution was removed in vacuo. The residue was dissolved in CH_2HI_2 (600 mL) and washed with 50% K_2CO_3 aqueous solution (2 x 200 mL). The combined aqueous solution was extracted with CH_2Cl_2 (2 x 200 mL). The combined organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, eluted with $CHCl_3$ – methanol (50 : 1), and the titled compound 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde (brown solid, 4.4 g, 40.7 %) and 6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde (orange solid, 1.7 g, 15.8 %) were obtained.

6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 6H), 1.78 (t, 2H, *J* = 6.9 Hz), 2.95 (t, 2H, *J* = 6.9 Hz), 3.71 (s, 2H), 7.46 (s, 1H), 9.83 (s, 1H).

6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde: ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 6H), 1.74 (t, 2H, *J* = 6.8 Hz), 2.97 (t, 2H, *J* = 6.8 Hz), 4.05 (s, 2H), 7.74 (s, 1H), 9.64 (s, 1H).

Step 5: (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (28 mL) solution of 6,6-dimethyl-5,6,7,8-tetrahydroimidazo [1,2-*a*]pyridine-2-carbaldehyde (4.55 g, 26 mmol) was added to the dry acetinitrile (152 mL) solution of $MgBr_2$ (5.22 g, 28 mmol) under a nitrogen atmosphere at room temperature, and then the mixture was stirred for 10 min. The dry THF (180 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (8.94 g, 23 mmol) was added and the mixture was cooled to –20 °C, and then triethylamine (7.8 mL, 56 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (0.29 g, 2.4 mmol) and acetic anhydride (4.4 mL, 47 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 h at 0 °C. Ethyl acetate (715 mL) was added to the reaction mixture, and then the organic layer was washed with

1mol/L Citric acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(6,6-dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia- 1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as brown amorphous solid.

Freshly activated Zn dust (53.6 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 282 mL) to the THF (192 mL) and acetonitrile (90 mL) solution of (5*R*)-6-[acetoxy-(6,6-dimethyl-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia- 1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 h at room temperature. The reaction mixture was cooled at 0 °C, and then the pH was adjusted to 7.6. Ethyl acetate (140 mL) was added to the reaction mixture, and then the mixture was filtered through a pad of Celite and the pad was washed with water (200 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The pH of the combined aqueous layer was adjusted to 8.1 and the mixture was concentrated to 584 g. 1mol/L NaOH was added to adjust pH to 8.2 and applied to Diaion HP-21 resin (420 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with 2.5 % (2 bed volume), 5 % (2 bed volume), 10 % (1 bed volume) and 20 % acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the crude (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2- ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt as a yellow amorphous solid (1.19 g).

The crude (5*R*),(6*Z*)-6-(6,6-Dimethyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyrizin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt was purified by the preparative HPLC (Mightysil RP-18 GP (5 μm), Kanto Chemical Co. Inc., 35 x 250 mm, 0.05 mol/L phosphate buffer (pH 7.2) : CH_3CN = 70 : 30, 20 mL/min.). The purified product was desalted by Diaion HP-21 resin (50 mL) column chromatography and the title compound was obtained 230 mg (2.8 %) as a yellow amorphous solid.

Mp 210°C (dec); ^{1}H NMR (D_2O) δ ; 0.91 (s, 3H), 0.93 (s, 3H), 1.63 (t, 2H, *J* = 6.8

Hz), 2.72 (t, 2H, J = 6.8 Hz), 3.60 (s, 2H), 6.44 (s, 1H), 6.90 (s, 1H), 6.91 (s, 1H), 7.19 (s, 1H).

Example 49

Preparation of (5*R*), (6*Z*)-6-(5,6-Dihydro-8-*H*-imidazo[2,1-c][1,4]thiazin-3-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

The dry acetonitrile (40 mL) solution of 5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazine-3-carbardehyde (813 mg) was added to the dry acetinitrile (40 mL) solution of $MgBr_2$ (2.2 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (80 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (2.1 g) was added, the mixture was cooled to –20 °C then triethylamine (1.7 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3.5 h at –20 °C and treated with 4,4-dimethylamino pyridine (64 mg) and acetic anhydride (0.9 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 14 h at 0 °C. 10% Citric acid aqueous solution (500 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 200 mL). The organic layer was washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography and eluted with CH_2Cl_2 – acetone (20 : 1) to obtain crude (5*R*)-6-[acetoxy-(5,6-dihydro-8*H*-imidazo[2,1-c][1,4]thiazin-3-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester as a brown solid.

The solid obtained above chromatography was dissolved in THF (11 mL). Freshly activated Zn dust (1.4 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 11 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction solution was filterd through a pad of Celite and the pad was washed with water (26 mL) and *n*-butanol (26 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 5 mL). The combined aqueous layer was concentrated to 18 g, 1mol/L NaOH was added to adjust pH to 7.3 and applied to Diaion HP-21 resin (20 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 5% acetonitrile aqueous solution. The combined active

fractions was concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid (81 mg).

Mp 145°C (dec); ^{1}H NMR (D_2O) δ 3.05-3.08 (m, 1H), 3.83 (s, 1H), 4.13-4.16 (m, 1H), 6.37 (s, 1H), 6.91 (s, 1H), 7.01 (s, 1H), 7.04 (s, 1H); IR (KBr) 3371, 1770, 1672, 1613 cm^{-1}; λ^{max} (H_2O) 314 nm.

Example 50

Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 3-Oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide

Conc. HCl (15 mL) and $NaNO_2$ (16.6 g) were added to the H_2O (166 mL) solution of *L*-thioproline (24.3 g) under a nitrogen atmosphere at 0 °C and stirred for 2 h. The solution was extracted with CH_2Cl_2, organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude N-nitroso compound as a yellow solid.

Trifluoroacetic anhydride (5.0 mL) was added to the THF (350 mL) solution of crude N-nitroso thioproline under a nitrogen atmosphere at 0 °C and stirred for 5 h at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (1 : 1). The titled compound was obtained as a pale brown solid (4.0 g, 15.1%).

^{1}H NMR ($CDCl_3$) δ: 4.04 (t, 2H, *J* = 1.7 Hz), 5.40 (t, 2H, *J* = 1.7 Hz).

Step 2: Preparation of 4*H*-5-Thia-1,6a-diazapentalen-2-carboxylic acid ethylester

Ethyl propiolate (3.1 mL) was added to the *o*-xylene (130 mL) solution of 3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-5-thia-1-aza-6a-azonio-3a-pentalenide (4.0 g) under a nitrogen atmosphere and refluxed for 19 h. The solution was cooled to room temperature and concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with *n*-hexane - AcOEt (4 : 1). The titled compound was obtained as a yellow solid (2.7 g, 49.3%), and 4*H*-5-thia-1,6a-diazapentalen-3-carboxylic acid ethylester was obtained as pale yellow crystals (1.2 g, 21.7%).

^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, *J* = 7.1 Hz), 4.11 (d, 2H, *J* = 2.1 Hz), 4.40 (q, 2H, *J* = 7.1 Hz), 5.24 (t, 2H, *J* = 1.6 Hz), 6.61 (s, 1H).

Step 3: Preparation of (4*H*-5-Thia-1,6a-diazapentalen-2-yl)methanol

$LiBH_4$ (cont. 90%) (459 mg) was added to the ether (126 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carboxylic acid ethylester (2.5 g) and MeOH (0.77 mL) under a nitrogen atmosphere at room temperature, then refluxed for 1.5 h. The mixture was quenched with 1 mol/L HCl (25 mL) and stirred for 1 h at room temperature. The mixture was neutralized by saturated sodium hydrogen carbonate solution and separated. The aqueous layer was extracted with dichloromethane (10 x 25 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with AcOEt. The titled compound was obtained as a pale yellow solid (1.7 g, 87.9%).

^{1}H NMR ($CDCl_3$):δ 2.95 (t, 1H, *J* = 5.6 Hz), 4.07 (s, 2H), 4.62 (d, 2H, *J* = 5.1 Hz), 5.13 (t, 1H, *J* = 1.6 Hz), 6.04 (s, 1H).

Step 4: Preparation of 4*H*-5-Thia-1,6a-diazapentalen-2-carbaldehyde

The dry dichloromethane (8 mL) solution of dimethylsulfoxide (2.2 mL) was added dropwise to the dry dichloromethane (110 mL) solution of oxalyl chloride (2.0 mL) at -78°C. The reaction mixture was stirred for 15 min at the same temperature. The dry dichloromethane (40 mL) solution of (4*H*-5-thia-1,6a-diazapentalen-2-yl)methanol (1.7 g) was added dropwise to the reaction mixture at -78°C, and stirring was continued for an additional 15 min. The reaction mixture was allowed to warm to -45°C and stirred for 1 h. Triethylamine (11.3 mL) was added dropwise and the reaction mixture was allowed to warm to 0°C. After 20min, saturated ammonium chloride solution (50 mL) and water (100 mL) were added and separated. The aqueous layer was extracted with AcOEt (3 x 150 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with hexane – AcOEt (1 : 1). The titled compound was obtained as a yellow solid (1.7 g, quant.).

^{1}H NMR ($CDCl_3$) δ 4.13 (s, 2H), 5.26 (d, 2H, *J* = 1.4 Hz), 6.59 (s, 1H), 9.90 (s, 1H).

Step 5: Preparation of (5*R*)(6*Z*)-7-Oxo-6-(4*H*-5-thia-1,6a-diazapentalen-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

The dry acetonitrile (92 mL) solution of 4*H*-5-thia-1,6a-diazapentalen-2-carbaldehyde (1.7 g) was added to the dry acetinitrile (92 mL) solution of $MgBr_2$ (5.0 g) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 min. The dry THF (184 mL) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3-carboxylate (4.3 g) was added and the mixture was cooled to –20 °C then triethylamine (7.4 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 h at –20 °C and treated with 4-dimethylamino pyridine (138 mg) and acetic anhydride (2.1 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The 1mol/L Citric acid aqueous solution (1000 mL) was added to the reaction mixture and the aqueous layer was extracted with ethyl acetate (3 x 400 mL). The combined organic layers were washed with water, saturated sodium hydrogen carbonate and brine, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as a brown amorphous.

Freshly activated Zn dust (19.3 g) was added rapidly with 0.5 mol/L phosphate buffer (pH 6.5, 100 mL) to the THF (100 mL) solution of crude (5*R*)-6-[acetoxy-(4*H*-5-thia-1,6a-diazapentalen-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2.5 h at room temperature. The reaction solution was filtered through a pad of Celite and the pad was washed with water (200 mL) and *n*-butanol (200 mL). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/L phosphate buffer (pH 6.5, 2 x 50 mL). The combined aqueous layers were concentrated to 90 g, 1mol/L NaOH was added to adjust pH to 8.0 and applied to Diaion HP-21 resin (180 mL, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water and then 15% acetonitrile aqueous solution. The combined active

fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a yellow amorphous solid (634 mg, 17.4%, pH 7.25).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ4.00 (s, 2H), 5.09 (s, 2H), 6.14 (s, 1H), 6.36 (s, 1H), 6.91 (s, 1H), 6.92 (s, 1H); IR (KBr) 3381, 1752, 1683, 1600, 1558 cm^{-1}; λ^{max} (H_2O) 292, 196 nm.

Example 51

Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 3-Oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-4-thia-1-aza-6a-azonio-3a-pentalenide

To a suspension of thiazolidine-2-carboxylic acid (39.9 g, 0.30 mol) in 1,000 ml of acetic acid was added a solution of 31.0 g (0.45 mol) of sodium nitrite in 500 ml of water over 13 minutes at room temperature and stirred for 5 hours. The reaction solution was concentrated under reduced pressure. Acetone (500 ml) was added to the residue and the precipitate was filtered through a pad of Celite. The pad was washed with acetone (500 ml). The filtrate was concentrated under reduced pressure to dryness and crude 3-nitrosothiazolidin-2- carboxylic acid was obtained as a yellow solid.

To a solution of crude 3-nitrosothiazolidin-2- carboxylic acid in 600 ml of dry tetrahydrofuran was added trifluoroacetic anhydride (189.6 g, 0.90 mol) over 20 minutes under a nitrogen atmosphere at 0 °C and stirred for 19 hours at 0 °C. The solution was concentrated under reduced pressure. The residue was applied to a silica-gel column chromatography, and then the column was eluted with *n*-hexane – ethyl acetate (1 : 1). The titled compound was obtained as a pale brown crystal (19.2 g, 44.5 %).

^{1}H NMR ($CDCl_3$) δ 3.98 (t, 2H, J = 7.7 Hz), 4.65 (t, 2H, J = 7.7 Hz).

Step 2: Preparation of 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3- dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester

Ethyl propiolate (20.3 ml, 0.20 mol) was added to an *o*-xylene (600 ml) solution of 3-oxo-3a, 4-dihydro-3*H*, 6*H*-2-oxa-4-thia-1-aza-6a-azonio-3a-pentalenide (19.2 g, 0.13 mol) under a nitrogen atmosphere and refluxed for 21 hours. The solution was cooled to room temperature and concentrated under reduced pressure. The residue

was applied to a silica gel column chromatography, and then the column was eluted with *n*-hexane – ethyl acetate (2 :21 to 1 : 1). The mixture of 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3- dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester was obtained as a brown oil in the ratio of 1:1.5 respectively. (21.2 g, Yield: 80.0 %).

2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester; ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, *J* = 7.1 Hz), 3.82 (t, 2H, *J* = 7.5 Hz), 4.39 (q, 2H, *J* = 7.1 Hz), 4.42 (t, 2H, *J* = 7.5 Hz), 6.52 (s, 1H).

2,3- dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.1 Hz), 3.85 (t, 2H, *J* = 7.8 Hz), 4.28 (q, 2H, *J* = 7.1 Hz), 4.39 (t, 2H, *J* = 7.8 Hz), 7.87 (s, 1H).

Step 3: 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde and 2,3-dihydro-pyrazolo[5,1-*b*]thiazol-7-carbaldehyde

To the mixture [21.2 g (0.11 mol)] of 2,3-dihydropyrazolo[5,1-*b*]thiazol-6-carboxylic acid ethyl ester and 2,3-dihydropyrazolo[5,1-*b*]thiazol-7-carboxylic acid ethyl ester in 540 ml of dry tetrahydrofuran was added $LiAlH_4$ (4.05 g, 0.11 mol) under a nitrogen atmosphere at 0 °C, and then stirred for 2.5 hours at room temperature. The mixture was quenched with water (15 ml) and the precipitate was filtered through a pad of Celite. The pad was washed with water (100 ml) and tetrahydrofuran (500 ml). The filtrate was concentrated under reduced pressure, and then water (150 ml) was added. The aqueous layer was extracted with dichloromethane (15 x 250 ml). The combined organic layers were dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and a mixture of (2,3-dihydropyrazolo[5,1-*b*]thiazol-6-yl) methanol and (2,3-dihydropyrazolo[5,1-*b*]thiazol-7-yl) methanol was obtained as pale brown oil (15.5 g).

To the mixture [15.5 g (0.10 mol)] of (2,3-dihydropyrazolo[5,1-*b*]thiazol-6-yl) methanol and (2,3-dihydropyrazolo[5,1-*b*]thiazol-7-yl) methanol in 500 ml of chloroform was added activated MnO_2 (77.7 g) under a nitrogen atmosphere at room temperature, and then refluxed for 3 hours. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column chromatography, and then the column was eluted with hexane-ethyl acetate (2 : 1 to 1 : 1). The titled compound 2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde

was obtained as a yellow crystal (2.50 g, 15.2 %) and _2,3-dihydro- pyrazolo[5,1-*b*]thiazol-7-carbaldehyde

was obtained as a pale brown solid (5.57 g, 33.8)

2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde; ^{1}H NMR ($CDCl_3$) δ 3.86 (t, 2H, *J* = 7.5 Hz), 4.45 (t, 2H, *J* = 7.5 Hz), 6.50 (s, 1H), 9.83 (s, 1H).

2,3-dihydro- pyrazolo[5,1-*b*]thiazol-7-carbaldehyde ; ^{1}H NMR ($CDCl_3$) δ 3.92 (t, 2H, *J* = 7.9 Hz), 4.40 (t, 2H, *J* = 7.9 Hz), 7.91 (s, 1H), 9.76 (s, 1H).

Step 4: Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]thiazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

A dry acetonitrile (19 ml) solution of 2,3-dihydropyrazolo[5,1-*b*]thiazol-6-carbaldehyde (2.50 g, 16.2 mmol) was added to a dry acetinitrile (106 ml) solution of $MgBr_2$ (3.67 g, 19.9 mmol) under a nitrogen atmosphere at room temperature then the mixture was stirred for 10 minutes. A dry tetrahydrofuran (125 ml) solution of *p*-nitrobenzyl (5R, 6S)-6-bromopenem-3- carboxylate (6.23 g, 16.2 mmol) was added and the mixture was cooled to –20 °C then triethylamine (5.4 ml, 38.7 mmol) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 3 hours at –20 °C and treated with 4-dimethylamino pyridine (198 mg, 1.62 mmol) and acetic anhydride (3.1 ml, 32.9 mmol) in one portion. The reaction mixture was warmed to 0 °C and stirred for 16 hours at 0 °C. Ethyl acetate (500 ml) was added to the reaction mixture and then the organic layer was washed with 1mol/l citric acid aqueous solution, saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the crude (5*R*)-6-[acetoxy-(2,3-dihydropyrazolo [5,1-*b*]thiazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester was obtained as a brown amorphous solid.

Freshly activated Zn dust (37.4 g) was added rapidly with 0.5 mol/l phosphate buffer (pH 6.5, 196 ml) to tetrahydrofuran (134 ml) and acetonitrile (62 ml) solution of (5*R*)-6-[acetoxy-(2,3-dihydropyrazolo [5,1-*b*]thiazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid *p*-nitrobenzyl ester. The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 1.5 hours at room temperature. The reaction mixture was cooled at 0 °C, and then the

pH was adjusted to 8.0. Ethyl acetate (100 ml) was added to the reaction mixture. The mixture was filtered through a pad of Celite and the pad was washed with water (300 ml). The aqueous layer was separated and then the organic layer was extracted with 0.5 mol/l phosphate buffer (pH 6.5, 2 x 50 ml). The pH of the combined aqueous layer was adjusted to 8.0 and the mixture was concentrated to 426 g. The concentrate was adjust pH to 8.0 and applied to Diaion HP-21 resin (540 ml, Mitsubishi Kasei Co. Ltd.) column chromatography. After adsorbing, the column was eluted with water (1 bed volume) and then 5 % (2 bed volume), 10 % (2 bed volume) and 20 % acetonitrile aqueous solution. The combined active fractions were concentrated under high vacuum at 35°C and lyophilized to give the title compound as a orange amorphous solid (2.09 g, 39.2 %, pH 7.10).

Mp 150 °C (dec); ^{1}H NMR (D_2O) δ 3.75 (t, 2H, *J* = 7.5 Hz), 4.27 (t, 2H, *J* = 7.5 Hz), 6.00 (s, 1H), 6.34 (s, 1H), 6.85 (s, 1H), 6.94 (s, 1H); IR (KBr) 3392, 1755, 1596, 1554 cm^{-1}; λ^{max} (H_2O) 290, 223 nm.

Example 52

Preparation of (5*R*)(6*Z*)-6-(2,3-Dihydropyrazolo[5,1-*b*]oxazol-6-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (10.34 g, 0.66 mol) and 36.62 g of potassium carbonate in 500 ml of acetonitrile was added 13.68 g of 1,2-dibromoethane, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. 5.80 g of the desired product was obtained (48%).

Step 2: Preparation of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-methanol:

To the stirred solution of ethyl2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carboxylate (5.47 g, 35 mmol) of in 100 ml of THF was added 1.05 g of lithium borohydride and 1.54 g of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1

hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 2.68 g of the desired product (65%).

Step 3: Preparation of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carbaldehyde:

To the stirred solution of 2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-methanol (2.60 g, 18.5 mmol) in 60 ml of CH_3Cl was added 12.9 g of MnO_2. Th suspension was refluxed for 1.5 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow oil. The product was purified by chromatography. 2.15 g of the product was obtained (84.3%).

Step 4: 4-Nitrobenzy (5R)-6-[(acetyloxy)(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-yl) -)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2,3-dihydropyrazolo[5,1-b][1,3]oxazole-6-carbaldehyde (607 mg, 4.3 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54 g, 4.6 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g , 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 1.9 g, 81%; M+H 566.

H-NMR($CDCl_3$) 8.24(2H, d, J= 6.6 Hz), 7.60(2H, d, J= 6.6Hz), 7.44(1H, s), 6.34(1H, s), 6.23(1H, s), 5.56(1H, s), 5.44(1H, d, J=10.2 Hz), 5.27(1H, d J=10.2Hz), 5.04(2H, m),

4.30(2H, m), 2.10(3H, s).

Anal.Calcd. for $C_{21}H_{17}BrN_4O_8S$: C, 44.61, H, 3.03, N, 9.91

Found: C, 45.00, H, 3.14, N, 9.53

Step 5: (5R,6Z)-6-(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-ylmethylene}-7oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt:

4-Nitrobenzy-6-[(acetyloxy)(2,3-dihydropyrazolo[5,1-b][1,3]oxazol-6-yl) -)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (700 mg, 1.2 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 276 mg, 73%; as yellow amorphous solid; (M+H+Na)314. .

^{1}H-NMR(D_2O); δ 6.97(1H, s), 6.95(1H, s), 6.46(1H, s), 5.56(1H, s) 5.07(2H, d, J= 6.3 Hz), 4.30(2H, t, J=6.3 Hz).

Example 53

Preparation of (5R,6Z)-6-[(5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2-carboxylic acid (E+Z Isomers mixture,Sodium salt)

Step 1: 5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde:

To a cold (0^0 C) suspension of 1.5 g.(7.4 mmol) of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde hydrochloride in 50 mL methylene chloride, under N_2 atm., dry conditions, was added dropwise under stirring 2.6 mL (2.5 eqs) of triethylamine. RM stirred for 30 min at 0^0 C. and a solution of 0.7 g.(8.1 mmol,1.1 eqs) of acetyl chloride in 15 mL methylene chloride was dropwise added, RM allowed to reach RT and stirred for 3 hours. Filtered trough a celite pad, filtrate washed with 3x 50 mL water, dried,

evaporated, gave 1.1g.(71.4 %) of the title compound, viscous oil, $(M+H)^+$ 210.3.

Step 2: Preparation of 4-nitrobenzyl(5R)-6-[(acetyloxy)(5-acetyl-4,5,6,7-tetrahydrotieno[3,2-c] pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2carboxylate

5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carbaldehyde (540 mg, 2.57 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (950 mg, 2.5 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g, 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 870 mg, 53%; m.p. 46- 48°C; $(M+H)^+$ 637.6.

1HNMR(CDCl3): δ 2.15(t,6H);2.8-3.0(m,2H);3.7-3.9(m,2H);4.58-4.68(m,2H);5.30-5.45(dd,2H);5.85(d,1H);6.71(s,1H);6.95(s,1H);7.35-7.45(d,1H); 7.60(dd,2H); 8.25(dd,2H).

Step 3: (5R,6Z)-6-[(5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)methylene]-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2-carboxylic acid (E+Z Isomers mixture,Sodium salt)

A solution of 0.77g.(1.21 mmol, 4-nitrobenzyl(5R)-6-[(acetyloxy)(5-acetyl-4,5,6,7-tetrahydrotieno[3,2-c] pyridin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-2-ene-2carboxylate in 40 mL THF and 40 mL phosphate buffer solution (pH=6.36) was hydrogenated at 40 psi for 3 hours in the presence of 0.4g. Palladium on Carbon 10% catalyst. Reaction mixture was filtrated through celite pad, filtrate adjusted to pH=8.0, concentrated in vacuo, residue purified on a reverse-phase column (amberlite), using

5%..10% ACN/water mixture as solvent, gave 0.107g.(23%) of the title compound, reddish crystals, m.p.362.4^0 C, $(M+H)^+$ 409.5.
1H NMR:δ 2.08 (s,3H);2.80-2.95 (m,1H);3.74(m,2H);3.98-4.06(d,2H)6.32-6.42 (s,1H); 6.50-6.60(s,1H);6.98-7.20 (s,1H);7.30-7.40 (s,1H).

Example 54

Preparation of (5*R*,6*Z*)-6-(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

Step 1: 4-Nitrosomorpholine-3-carboxylic acid

To a solution of morpholine-3-carboxylic acid (6.96 g, 52 mmol) in water (20 ml), at 0 °C under nitrogen, was added concentrated hydrochloric acid (4 ml), followed by sodium nitrite (5.0 g, 72 mmol) in small portions. The mixture was stirred at 0 °C for 1 hr, and then concentrated under vacuum at 30 to 35 °C. The residue was stirred with 200 ml of acetone and filtered. The filtrate was evaporated and the residue treated with 50 ml of THF and concentrated. The process was repeated with 2x50 ml of THF to give 11.87 g of light yellow foam; MS (ESI) *m/z* 159.2 (M-H).

Step 2: 6,7-Dihydro-4*H*-[1,2,3]oxadiazolo[4,3-c][1,4]oxazin-8-ium-3-olate

The crude 4-nitrosomorpholine-3-carboxylic acid (11.0 g) from step 1 was dissolved in THF (250 ml) and cooled to 0 °C. A solution of trifluoroacetic anhydride (7.4 ml, 52 mmol) in THF (20 ml) was added with stirring over 10 min. The resulting mixture was stirred at 0 °C for 5 hr, and warmed to room temperature for 16 hr. The solvent was evaporated and the residue was diluted with 250 ml of ethyl acetate and stirred with 30 g of anhydrous potassium carbonate. The mixture was filtered through a pad of silica gel and the filtrate evaporated. The residue was washed with a mixture of ethyl acetate-ether to give 3.80 g of a white solid; mp 132-133 °C; MS (ESI) *m/z* 143.1 (M+H).

Step 3: Ethyl 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carboxylate

To a partial solution of 6,7-dihydro-4*H*-[1,2,3]oxadiazolo[4,3-c][1,4]oxazin-8-ium-3-olate (3.41 g, 24 mmol) in o-xylene (80 ml), was added ethyl propiolate (2.7 ml, 26 mmol). The mixture was stirred at 140 °C for 3 hr. An additional 2.0 ml (19 mmol) of ethyl propiolate

was then added and the mixture was stirred at reflux for 18 hr. The final solution was evaporated under vacuum, and the residue was dissolved in a mixture of methylene chloride and hexanes (1:5). The solution was passed through a pad of silica gel and the filter pad was eluted with methylene chloride-hexanes, followed by ethyl acetate. The ethyl acetate eluent was evaporated and the residue washed with hexanes to give 4.10 g of a white solid; mp 63 °C; MS (ESI) *m/z* 197.1 (M+H).

Step 4: 6,7-Dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethanol

To a solution of ethyl 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carboxylate (1.57 g, 8.0 mmol) in methylene chloride (30 ml) was added 24 ml of a 1.0 M solution of diisobutylaluminum hydride in methylene chloride at 0 °C, under nitrogen. After stirring for 0.5 hr at 0 °C, the mixture was warmed to room temperature for 2 hr. It was then treated with 30 ml of saturated ammonium chloride solution and extracted with ethyl acetate. The organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to give 1.27 g of a colorless oil; MS (ESI) *m/z* 155.3 (M+H).

Step 5: 6,7-Dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde

To a solution of 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethanol (1.08 g, 7.0 mmol) in 1,2-dichloroethane (30 ml) was added 5.4 g of activated manganese dioxide at room temperature with stirring. The mixture was heated to 60 °C for 1 hr and then stirred at room temperature for 16 hr. The final mixture was filtered through a column of silica gel topped with celite. The filter pad was eluted with methylene chloride, followed by ethyl acetate. The ethyl acetate eluent was evaporated and the residue triturated with to give 0.81 g of a white solid; mp 91 °C; MS (ESI) *m/z* 153.2 (M+H).

Step 6: 4-Nitrobenzyl (5*R*)-6-[(acetyloxy)(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

To a solution of $MgBr_2$ (0.94 g, 5.1 mmol) in acetonitrile (25 ml) under nitrogen was added 6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazine-2-carbaldehyde (0.26 g, 1.7 mmol) at room temperature with stirring. A solution of (5R,6S)-6-bromo-7-oxo-4-thia-1-

azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester (0.58 g, 1.5 mmol) in THF (25 ml) was then added, and the mixture was cooled to –20 °C. Triethylamine (0.71 ml, 5.1 mmol) was introduced, and the mixture was stirred at –20 °C in the dark for 5 hr. It was then treated with acetic anhydride (0.6 ml, 6.0 mmol), and 4-N,N-dimethylaminopyridine (24 mg, 0.2 mmol), and kept at 0 °C for 18 hr. The mixture was concentrated and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with 5% citric acid, saturated sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated. The crude material was chromatographed with silica gel (EtOAc-CH2Cl2/1:5) to give 0.77 g of a white foam; MS (ESI) *m/z* 578.9 (M+H).

Step 7: (5*R*,6*Z*)-6-(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

To a solution of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(6,7-dihydro-4*H*-pyrazolo[5,1-c][1,4]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.35 g, 0.6 mmol) in THF (20 ml), under nitrogen, was added 20 ml of a phosphate buffer solution (0.5M, pH 6.5), and 120 mg of 10% Pd/C. The mixture was hydrogenated at 40-50 psi for3 hr, and then filtered through Celite. The filter pad was washed with THF, and the filtrate was extracted with ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was washed with ether to give 0.09 g of a yellow solid; HRMS: calcd for $C_{13}H_{11}N_3O_4S$, 305.0470; found (ESI+), 306.05434; ^{1}H NMR (DMSO-d_6) δ 4.07-4.09 (t, 2H), 4.13-4.17 (t, 2H), 4.82 (s, 2H), 6.36 (s, 1H), 6.55 (s, 1H), 7.17 (s, 1H), 7.55 (s, 1H), 12.80 (bs, 1H).

Example 55

Preparation of (5*R*)(6*Z*)-6-(6,7-5H--Dihydropyrazolo[5,1-*b*]oxazin-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (10.34 g, 0.66 mol) and 36.62 g of potassium carbonate in 500 ml of acetonitrile was added 14.7 g of 1,3-dibromopropane, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The

filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. 8.80 g of the desired product was obtained (68%), m.p. 44-46^{0}C $(M+H)^+$ 197.1.

Step 2: Preparation of 2,3-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl-methanol:

To the stirred solution of 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carboxylate: (4.0 g, 20 mmol) of in 100 ml of THF was added 0.71 g of lithium borohydride and 1.03 g of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 2.08 g of the desired product (67%); (M+H) 155.

Step 3: Preparation of 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carbaldehyde:

To the stirred solution of 2,3-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl-methanol (2.08 g, 13.5 mmol) in 60 ml of CH_3Cl was added 9.38 g of MnO_2. Th suspension was refluxed for 2 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow oil. The product was purified by chromatography. 2.15 g of the product was obtained (78%).

Step 4: 4-Nitrobenzy(5R)-6-[(acetyloxy)(6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-2-carbaldehyde (330 mg, 2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (0.794 g, 2.2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04

mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 0.76 g, 65%; M+H 579.

Step 5: (5*R*)(6*Z*)-6-(6,7-5H--Dihydropyrazolo[5,1-*b*]oxazin-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4-Nitrobenzy(5R)-6-[(acetyloxy)(6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (350 mg, 0.6 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Dried. Yield: 103 mg, 52%; as yellow amorphous solid; (M+H+Na)327.
^{1}H-NMR(D_2O); δ 6.97(1H, s), 6.93(1H, s), 6.47(1H, s), 5.65(1H, s) 4.28(2H, m), 4.10(2H,m), 2.21 (2H,m).

Example 56

Preparation of (5R,6Z)-6-[(5-methylimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Step 1: Ethyl 5-methylimidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl 5-methylimidazo[2,1-b]-benzthiazole-2-carboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from 4-methly-2-amino benzothiazole (8.0 g, 48.7 m.mol) and ethyl bromopyruvate (14.0 g, 71.7 mmol), 6.0 g (45% Yield) of ethyl 5-methylimidazo[2,1-b]-benzthiazole-2-carboxylate was isolated as a brown solid. (M+H) 261.

Step 2: 5-methyl imidazo[2,1-b]-benzthiazole-2-methanol:

5-methyl imidazo[2,1-b]-benzthiazole-2-methanol was prepared according to the procedure outlined in Example 1, (Step 2). Starting from ethyl 5-methylimidazo[2,1-b]-benzthiazole-2-carboxylate (5.2 g, 20 mmol) and $LiAlH_4$ solution (22 ml, 0.5 M solution in THF), 3 g (69% yield) of the alcohol derivative was isolated as a brown solid. (M+H) 219.

Step 3: 2-Formyl-5-methylimidazo[2,1-b]-benzthiazole:

2-Formyl-5-methylimidazo[2,1-b]-benzthiazole was prepared according to the procedure outlined in Example 1, (Step 3). Starting from 5-methyl imidazo[2,1-b]-benzthiazole-2-methanol (2.0 g 9.1 mmol) in methylene chloride/ DMF(300 mL: 50 mL) and active MnO_2 (12 g, excess), 700 mg (35% Yield) of the aldehyde derivative was isolated as brown solid. (M+H) 217.

Step 4: 4-Nitrobenzyl-6-[(acetyloxy) (5-methylimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-5-methylimidazo[2,1-b]-benzthiazole (432 mg, 2.0 mmol) and the dry THF solution (40 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$:etherate (1.3 g, 5mmol) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to a

silica gel column, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 270 mg, 20%; M+H 644.

Step 5: (5*R*),(6*Z*)-6-[(5-methylimidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy) (5-methylimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (400 mg, 0.62 mmol) was dissolved in THF (17 mL) and acetonitrile (36 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 N NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The precipitate was filtered and washed with H_2O, MeCN, acetone to give the title compound. Yield: 60 mg, 24%; as yellow crystals; mp 192; M+Na 392.

^{1}H NMR (DMSO-d_6) δ 2.1 (s, 3H), 6.53(s, 2H), 7.1(s, 1H), 7.34-7.36 (m, 2H), 7.85(m, 1H), 8,58 (s, 1H).

Example 57

Preparation of (5R,6Z)-6-[(7-fluoroimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Step 1: Ethyl 7-fluoroimidazo[2,1-b]-benzthiazole-2-carboxylate:

Ethyl 7-fluoro-imidazo[2,1-b]-benzthiazole-2-carboxylate was prepared according to the procedure as outlined in Example 1, (Step 1). Starting from 6-fluoro-2-amino benzothiazole (10.0 g, 59.5 m.mol) and ethyl bromopyruvate (17.4 g, 89.2 mmol), 3.0 g (19% Yield) of ethyl 7-fluoro-imidazo[2,1-b]-benzthiazole-2-carboxylate was isolated as a brown semi-solid. (M+H) 265.

Step 2: 7-fluoro- imidazo[2,1-b]-benzthiazole-2-methanol:

7-Fluoro-imidazo[2,1-b]-benzthiazole-2-methanol was prepared starting from Ethyl 7-fluoro-imidazo[2,1-b]-benzthiazole-2-carboxylate (2.64 g, 0.01 mol) and $LiBH_4$ (50 mg) in THF at refluxing temperature for 2 hrs. at the end, reaction mixture was quenched with ice cold water and acidified with 10 N. HCl. Reaction mixture was stirred for 1 hr and nuetralized with K_2CO_3. The separated residue was extracted with chloroform: methanol (3:1) and dried over anhydrous $MgSO_4$. It was filtered and concentrated. The crude reaction mixture was found to be pure and taken to next step with out any purification. Yeild: 1.5 g (68%) Semi solid; M+H 223.

Step 3: 2-Formyl-7-fluoro-imidazo[2,1-b]-benzthiazole:

2-Formyl-7-fluoro-imidazo[2,1-b]-benzthiazole was prepared according to the procedure outlined in Example 1, (Step 3). Starting from 7-fluoro-imidazo[2,1-b]-benzthiazole-2-methanol (1.5 g 6.7 mmol) in methylene chloride/ DMF(300 mL: 50 mL) and active MnO_2 (12 g, excess), 1.1 g (78% Yield) of the aldehyde derivative was isolated as brown solid. (M+H) 221.

Step 4: 4-Nitrobenzyl-6-[(acetyloxy) (7-fluoro-midazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-7-fluoro-imidazo[2,1-b]-benzthiazole (500 mg, 2.3 mmol) and the dry THF solution (40 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (875 mg, 2.3 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$:etherate (1.3 g, 5mmol) under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo

isomers were taken to next step. Pale yellow amorphous solid; Yield: 330 mg, 22%; M+H 649.

Step-5: (5*R*),(6*Z*)-6-[(7-fluoro-imidazo[1,2-*b*][1,3]benzothiazol-2-ylmethylene)] -7-oxo-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy) (7-fluoro-imidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (710 mg, 1.07 mmol) was dissolved in THF (17 mL) and acetonitrile (36 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 1 N NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The precipitate was filtered and washed with H_2O, MeCN, acetone to give the title compound. Yield: 80 mg, 19%; as yellow crystals; mp 200 (dec); M+Na 396.

^{1}H NMR (DMSO-d_6) δ 6.53(s, 1H), 6.63(s, 1H), 7.1(s, 1H), 7.45 (t, 1H), 8.04 (m, 1H), 8,13-8.10 (m, 1H), 8.61 (s,1H).

Example 58

Preparation of (5*R*),(6*Z*)-6-(5,8-dihydro-6H-imidazo[2,1-b]pyrano[4,3-d][1,3]thiazol-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of ethyl 5,8-dihydro-6H-imidazo[2.1-b]pyrano[4,3-d][1,3]thiazole-2-carboxylate

A mixture of tetrahydro-4H-pyran-4-one (5.0 g, 50 mmol) in CCl_4 (100 ml) at 0°C, SO_2Cl_2 (7.4 g, 55 mmol) was slowly added. After the addition, reaction mixture was stirred at room temperature for 4 hrs and carefully quenched with ice cold water. Recation mixture was washed well and dried over anhydrous $MgSO_4$. The organic layer was filtered and concentrated. The colurless oil obtained was diisoolved in THF/EtOH containing thiourea (4.0 g, 52 mmol) and refluxed for 8 hrs. At the end, reaction mixture was cooled to room temperature and the separated, 6,7-dihydro-4H-pyrano[4,3-

d][1,3]thiazol-2-amine hydrochloride white solid was filtered. Yield. 4.5 g (47%); M.Pt. 115°C, (M+H) 157.

To a stirred mixture of , 6,7-dihydro-4H-pyrano[4,3-d][1,3]thiazol-2-amine hydrochloride (4.0 g, 20.8 mmol) was dissolved in anhydrous ethanol (100 ml) and sodium methoxide (1.1 g, 21 mmol). This was stirred at room temperature for 30 minutes and to this ethyl bromopyruvate (10 .0 g) was added and stirred at room temperature for 2 hrs. Then it was refluxed for 48 hrs. At the end reaction mixture was cooled to room temperature and concentrated. The residue was extracted with chloroform and washed well with water. The product was purified by silica-gel column chromatography by eluting it with 50% ethyl acetae: hexane. Red semi-solid; Yield: 3.1 g, (59%) M+H 253.

The ester was reduced with $LiBH_4$ and the resultant alcohol was oxidized with active MnO_2. The aldehyde obtained was taken to next step.

Step 3: Preparation of 4-nitrobenzyl (5R)-6-[(acetyloxy)(5,8-dihydro-6H-imidazo[2,1-b][1,3]pyrano[4,3-d][1,3]thiazol-2-yl)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

2-Formyl-5,8-dihydro-6H-imidazo[2.1-b]pyrano[4,3-d][1,3]thiazole (208 mg, 1.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (400 mg, 1.1 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced

pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 400 mg, 62%; M.Pt. 78°C; M+H 636.

Step 4: Preparation of (5*R*),(6*Z*)-6-(5,8-dihydro-6H-imidazo[2,1-b]pyrano[4,3-d][1,3]thiazol-2-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5R)-6-[(acetyloxy)(5,8-dihydro-6H-imidazo[2,1-b][1,3]pyrano[4,3-d][1,3]thiazol-2-yl)-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (500 mg, 0.79 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 85 mg, 30%; as yellow crystals; mp 205°C; (M+H+Na) 383 .^{1}H NMR (DMSO-d_6) δ 2.8 (m, 2H), 4.0 (m,2H), 4.6(s,2H), 6.4 (s,1H), 6.5 (s,1H), 7.0 (s,1H), 8.1 (s,1H).

Example 59

Preparation of (5*R*),(6*Z*)-6-(imidazo[2,1-b]bebzothiazol-7-ylmethylene)-7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of imidazo[2,1-b][1,3]benzothiazol-7-ylmethanol:

A solution of ethyl imidazo[2,1-b][1,3]benzothizole-7-carboxylate (1.1 g, 4.5 mmol) in THF (50 ml) was slowly added to to a stirred solution of $LiBH_4$ (1 g) in THF (100 ml) at 0°C . The reaction mixture was refluxed for 2 hrs and cooled to room temperature. It was quenched with ice cold water andf carefully nuetralized with Con. HCl. The soltion was stirred at room temperature for 2 hrs and basified with K_2CO_3 (solid). At the end, reaction mixture was extracted with chloform: methanol (3:1) and dried over anhydrous

$MgSO_4$. It was filtered and concentrated. The product was pue enough and taken to next step with out purification. Brown solid. M.t. 75°C; (M+H) 205. Yield; 800 mg, (87%).

Step 2: Preparation of 7-fomyl- imidazo[2,1-b][1,3]benzothiazol:

Imidazo[2,1-b][1,3]benzothiazol-7-ylmethanol (700 mg, 3.4 mmol) obtained by the above mentioned process was oxidiazed with active MnO_2 (2 g) in $CH_2Cl_{2=}$ under refluxing condition. The reaction mixture was stirred for 6 hrs and cooled to room temperature. It was filtered and through celite and concentrated. The separated brown color solid was triturated with diethyl ether and filtered. It was found to be pure enough and taken to next step with out purification. Yield. 400 mg (58%); (M+H) 203.

Step 3: 4-Nitrobenzyl-6-[(acetyloxy) (imidazo[2,1-b][1,3]benzothiazol-7-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

7-fomyl- imidazo[2,1-b][1,3]benzothiazol (260 mg, 1.3 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (500 mg, 1.14 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (390 mg, 1.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 750 mg, 91%; M.pt. 82°C; M+H 630.

Step 4: 5*R*),(6*Z*)-6-(imidazo[2,1-b]bebzothiazol-7-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl-6-[(acetyloxy) (imidazo[2,1-b][1,3]benzothiazol-7-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (900 mg, 1.4 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and 0.5 *M* phosphate buffer (pH 6.5, 20 mL) and hydrogenated over Pd/C (10%) at 40 psi pressure for 6 hrs. The reaction vessel was covered with foil to exclude light. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was concentarted and the aqueous layer was washed with ethyl acetate. The aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 180 mg, 36%; as yellow crystals; mp 235^{0}C; (M+H+Na) 378.

^{1}H NMR (DMSO-d_6) δ 6.3 (s, 1H), 6.6 (s,1H), 7.1 (s, 1H), 7.52 (s, 1H), 8.1-8.5 (m,3H), 8.7 (s, 1H).

Example 60

Preparation of (5*R*),(6*Z*)-7-oxo-6-([1,3]thiazolo[3,2-a]benzimidazol-2-ylmethylene)-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of benzo[4,5]imidazo[2,1-b]thazole-2-carbaldehyde:

To a stirred solution of 2-mercapto benzimidazole (5.0 g, 33.3 mmol) and K_2CO_3 (4.59 g, 33.3 mmol) in anhydrous DMF (100 mL) bromomalonaldehyde (4.99 g, 33.3) was added and heated fo 8 hrs at 80°C. At the end, reaction mixture was concentrated to dryness and ice cold water was added.and nuetralzed with 1N HCl. The product was extarcted with chloroform and washed with water and dried over anhydrous $MgSO_4$. It was filterd and concentrated. The residue was taken in DMF/ acetic acid mixture (1:1) (100 ml) and heated at 120°C for 6 hrs. The reaction mixture was concentarted and extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentarted. The separated solid was triturated with diethyl ether and filtered. Yield: 4.2 g (62%); (M+H) 203.

Step 2: 4-Nitrobenzyl (5R)-6-[(acetyloxy) ([1,3]thiazolo[3,2-a]benzimidazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2- ne-2-carboxylate:

Benzo[4,5]imidazo[2,1-b]thazole-2-carbaldehyde (404 mg, 2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (772 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.65 g, excess)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 800 mg 63%; M.pt. 78°C; (M+H) 630.

Step 3: (5*R*),(6*Z*)-7-oxo-6-([1,3]thiazolo[3,2-a]benzimidazol-2-ylmethylene0-4-thia-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid:

4-Nitrobenzyl (5R)-6-[(acetyloxy) ([1,3]thiazolo[3,2-a]benzimidazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate: (630 mg, 1.0 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and 0.5 *M* phosphate buffer (pH 6.5, 20 mL) and hydrogenated over Pd/C (10%) at 40 psi pressure for 6 hrs. The reaction vessel was covered with foil to exclude light. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was concentarted and the aqueous layer was washed with ethyl acetate. The aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with

10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 190 mg, 50%; as yellow crystals; mp 240^{0}C (Dec); (M+H+Na) 378 .

^{1}H NMR (DMSO-d_6) δ 6.3 (s, 1H), 6.4 (s,1H), 6.6 (d, 2H), 7.29-7.39 (m, 2H), 7.69-7.73 (t,1H), 8.1-8.19 (m, 1H), 8.84 (s,1H).

Example 61

Preparation of (5*R*),(6*Z*)-6-(7,8-dihydro-6H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazol-2-ylmethylene)-7-oxo-6-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 7,9-dihydro-6H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazole-2-carbaldehyde:

To a stirred solution of 1,4,5,6-tetrahydrocyclopenta[c]pyrazole-3(H)-thione [Prepared by the procedure of T.takeshima, N. Oskada, E.Okabe and F. mineshima, J. Chem. Soc. Perkin. Trans. I, 1277-1279, (1975)] (5.3 g, 37.85 mmol) and K_2CO_3 (10.4 g, 75 mmol) in anhydrous DMF (100 mL) bromomalonaldehyde (5.7 g, 37.85) was added and heated fo 8 hrs at 80°C. At the end, reaction mixture was concentrated to dryness and ice cold water was added.and nuetralzed with 1N HCl. The product was extarcted with chloroform and washed with water and dried over anhydrous $MgSO_4$. It was filterd and concentrated. The residue was taken in DMF/ acetic acid mixture (1:1) (100 ml) and heated at 120°C for 6 hrs. The reaction mixture was concentarted and extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentarted. The product was purified by SiO_2 column chromatography by eluting it with 75% ethyl acetate: hexane. Yield: 2.2 g (30%); M.Pt. 112°C; (M+H) 193.

Step 2: 4-Nitrobenzyl-(5R)-6-[(acetyloxy) (7,8-dihydro-8H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

7,9-dihydro-6H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazole-2-carbaldehyde (576 mg, 3 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.16 g, 3 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$

(1.65 g, excess)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 1.5 g, 83%; M.pt. 69°C; (M+H) 620.

Step 3: (5*R*),(6*Z*)-6-(7,8-dihydro-6H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazol-2-ylmethylene)7-oxo-4-thia-1- azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-Nitrobenzyl-(5R)-6-[(acetyloxy) (7,8-dihydro-8H-cyclopenta[3,4]pyrazolo[5,1-b][1,3]thiazol-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (1.2 g, 1.9 mmol) was dissolved in THF (30 mL) and acetonitrile (30 mL) and 0.5 *M* phosphate buffer (pH 6.5, 30 mL) and hydrogenated over Pd/C (10%) at 40 psi pressure for 6 hrs. The reaction vessel was covered with foil to exclude light. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was concentarted and the aqueous layer was washed with ethyl acetate. The aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 420 mg, 38%; as yellow crystals; mp 190°C (Dec); (M+H+Na) 368 .

^1H NMR (DMSO-d_6) ^1H NMR (DMSO-d_6) δ 2.38 -2.42 (m, 2H), 2.69-2.89 (m, 4H), ,6.57 (s, 1H), 6.58 (s,1H), 7.36 (s, 1H), 8.53 (s,1H).

Example 62

Pr paration of (5*R*),(6*Z*)-7-oxo-6-(5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)- 4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of ethyl 5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazole-2-carboxylate.

A mixture of 2-chlorocyclohexanone (13.2 g, 100 mmol) and thiourea (8.0 g 101 mmol) was refluxed in ethanol: THF (1:2) for 16 hrs. The reaction mixture was cooled to room temperature and the separated white solid was filtered. (12.0 g separated) This was dissolved in anhydrous ethanol (100 ml) and sodium methoxide (3.3 g, 63 mmol). To this ethyl bromopyruvate (15 .0 g) was added and stirred at room temperature for 2 hrs. Then it was refluxed for 48 hrs. At the end reaction mixture was cooled to room temperature and concentrated. The residue was extracted with chloroform and washed well with water. The product was purified by silica-gel column chromatography by eluting it with 50% ethyl acetae: hexane. Red semi-solid; Yield: 6.2 g (39%); M+H 251.

The ester was reduced with $LiBH_4$ and the resultant alcohol was oxidized with active MnO_2. The aldehyde obtained was taken to next step.

Step 2: Preparation of 4-nitrobenzyl (5R)-6-[(acetyloxy)(5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]- 6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazole-2-carbaldehyde (412 mg, 2.0 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted

with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 980 mg, 77%; M+H 634.

Step 3: Preparation of (5*R*),(6*Z*)-7-oxo-6-(5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazol-2-ylmethylene)- 4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5R)-6-[(acetyloxy)(5,6,7,8-tetrahydroimidazo[2,1-b][1,3]benzothiazol-2-yl)methyl]- 6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (980 mg, 1.55 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28 mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 120 mg, 20%; as yellow crystals; mp 250^{0}C (Dec); (M+H+Na) 382 .^{1}H NMR (DMSO-d_6) δ 1.9 (m,2H), 2.5 (m, 2H), 3.2-3.4 (m, 4H), 6.6 (s, 1H), 7.1 (s, 1H), 7.5 (s, 1H), 8.1 (s, 1H).

Example 63

Preparation of (5*R*),(6*Z*)-8-[(9-methyl-9H-imidazo[1,2-a]benzimidazol-2-yl)methylene]-7-oxo- 4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Step 1: Preparation of 9-methyl-9H-imidazo[1,2-a]benzimidazole-2-carbaldehyde.

To stirred solution of $LiBH_4$ (1.79 g, 82 mmol) in THF at 0^{o}C, ethyl 9-methyl-9H-imidazo[1,2-a]benzimidazole-2-carboxylate (2.5 g, 10.3 mmol) was added drop wise. The reaction mixture was refluxed for 2 hrs and cooled to room temperature. Ti was carefully quenched with icve cold water and acidified with Con. HCl to pH 4. The reaction mixture was stirred at room temperature for 1 hr and basified with K_2CO_3. The

residue was extracted with chloroform;methanol (3:1) and dried over anhydrous $MgSO_4$. It was filtered and concentrated. Yield. 1.3 g (65%). (M+H) 202.

The resdue (1.3 g, 6.4 mmol) was oxidised with MnO_2 (5.0 g) in CH_2Cl_2 under refluxing condition. After the completion, reaction mixture was filtered and concentrated. It was purified by SiO_2 column chromatography by eluting it with 1:1 ethyl acetate: hexane. Brown solid. Yield. 330 mg (25%); (M+H) 200.

Step 2: Preparation of 4-nitrobenzyl (5R)-6-[(acetyloxy)(9-methyl-9H-imidazo[1,2-a]benzimidazole-2-)methyl]- 6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

9-methyl-9H-imidazo[1,2-a]benzimidazole-2-carbaldehyde. (330 mg, 1.65 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 330 mg, 31%; (M+H) 628.

Step 3: Preparation of (5*R*),(6*Z*)-8-[(9-methyl-9H-imidazo[1,2-a]benzimidazol-2-yl)methylene]-7-oxo- 4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5R)-6-[(acetyloxy)(9-methyl-9H-imidazo[1,2-a]benzimidazole-2-)methyl]- 6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate:

(1 g, 1.6 mmol) was dissolved in THF (20 mL) and acetonitrile (10 mL). Freshly activated Zn dust (5.2 g) was added rapidly with 0.5 *M* phosphate buffer (pH 6.5, 28

mL). The reaction vessel was covered with foil to exclude light. The reaction mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 140 mg, 23%; as yellow crystals; mp 220^{0}C (Dec); (M+H+Na) 375 .^{1}H NMR (DMSO-d_6) δ 3.4 (s,3H), 6.54 (s, 1H), 6.56 (s, 1H), 7.01 (s, 1H), 7.21 (t, 1H), 7.3 (t, 1H), 7.56 (d, 1H), 7.85 (d,1H), 8.1 (s,1H).

Example 64

Preparation of (5R,6Z)-7-oxo-6-(4H-thieno[2',3':4,5]thiopyrano[2,3-b]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (Sodium salt)

Step 1: 2,3 dihydro-4H-thiopyrano[2,3-b]pyridin-4-one:

A solution of 14 g. (61.6 mmol) 3-(3-Carboxy-2-pyridylthio)propionic Acid [prepared as described in lit.:J.Heterocyclic Chem.,**37**,379(2000)] and 15 g.(185 mmol,3 eqs) of anhydrous sodium acetate, in 200 mL. of acetic anhydride was refluxed (160^0 C) under stirring, N_2 atm, dry conditions, for 2 hours. Cooled, diluted with 300 mL of water,basified with 30% ammonium hydroxide solution to pH 8-9, extracted with 3x200 mL chloroform. Combined organics washed with 2x60 mL Sodium bicarbonate (satn.sol), water,dried, evaporated, gave 2.8g. (27%) of the title compound, reddish solid, m.p.66-8^0 C, $(M+H)^+$=166.2.

Step 2: 4-chloro-2H-thiopyrano[2,3b]pyridine-3-carbaldehyde:

A solution of 6.6g.(43 mmol,1 eq) of phosphorous oxychloride in 30 mL methylene chloride was dropwise added to 3.95g (43 mmol,1.25 eqs) of anhydrous dimetylformamide (0^0 C, stirring, N_2 atm, dry conditions) with such a rate to maintain temperature between 0-5^0 C; RM was stirred at RT for 2 hours, cooled to 0^0 C, and a

solution of 8.9 g.(54 mmol,1.25 eqs.) of 2,3 dihydro-4H-thiopyrano[2,3-b]pyridin-4-one in 30 mL of methylene chloride was dropwise added over a 20 min. period. RM stirred at RT for 2 hours, poured over crushed ice:sodium acetate 4:1 mixture, extracted with 4x 150 mL methylene chloride, combined organics washed with water, dried, evaporated, gave 7.76g (68%) of the title compound, brownish solid,m.p.56-8^0 C, $(M+H)^+$=212.6.

Step 3: Ethyl 4H-thieno[2'3':4,5]thiopyrano[2,3b]pyridine-2 carboxylate:

To a solution of 7.5g. (35 mmol, 1 eq.) of 4-chloro-2H-thiopyrano[2,3b]pyridine-3-carbaldehyde in 25o mL of methylene chloride were added (under stirring, N_2 atm, dry conditions): 4.7 g.(39 mmol,1.1 eqs) of ethyl mercaptoacetate, and 7.2 g. (71 mmol,2 eqs) of triethylamine in 30 mL of methylene chloride. RM was refluxed for 2 hours,quenched with 100 mL of water, organics separated, waters extracted with 4x150 mL of methylene chloride, combined organics dried, evaporated. Residue purified on a silicagel column, using hexane:ethyl acetate 3:1 as a solvent, gave 7.6g. (78%) of the title compound, yellow crystals, m.p. 113-5^0 C, $(M+H)^+$= 278.3.

Step 4: 4H-Thieno[2',3':4,5]thiopyrano[2,3b]pyridin-2-ylmethanol:

To a cold solution of 7.5g.(27 mmol) of Ethyl 4H-thieno[2'3':4,5]thiopyrano[2,3b]pyridine-2 carboxylate in 300 mL of dry tetrahydrofuran (0^0 C, N_2 atm,dry condition) was dropwise added 60 mL (60 mmol, 2.1 eqs) of 1M cold solution of Lithium Aluminum Hydride in tetrahydrofuran, and RM stirred at RT untill the SM disappeared (monitored by TLC/MS). Cooled to 0^0 C, RM was quenced with aquous 2N formic acid solution to neutral pH=8, and stirred at RT for 2 hours, filtered, filtrate extracted 4x 200 mL methylene chloride, combined organics dried, evaporated gave 6.0 g. (94%) of the desired compound, yellow crystals, m.p.112-4^0 C, $(M+H)^+$= 236.4.

Step 5: 4H-thieno[2',3':4,5]thiopyrano[2,3b]pyridin-2-carbaldehyde:

To a solution of 3.0 g.(12.8 mmol) of 4H-thieno[2',3':4,5]thiopyrano[2,3b]pyridin-2-ylmethanol in 200 mL of chloroform, was added 9.0 g.(80 mmol, 7 eqs) of activated manganese(IV)oxide, and RM refluxed under stirring, N_2 atm., for 12 hours. Filtered trough a celite pad, filtrate evaporated, and residue purified on a silicagel column, gave 2.5 g.(86%) of the title compound, yellow crystals, m.p. 93-5^0 C, $(M+H)^+$= 234.4.

Step 6: 4-nitrobenzyl(5R)-6-[(acetyloxy)(4H-thieno [2',3':4,5]thiopyrano [2,3b]pyridin-2-yl) methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-ene-2carboxylate

In a sealed dry r.b. flask, flushed with N_2, were added: 4H-thieno[2',3':4,5]thiopyrano[2,3b]- pyridin-2-carbaldehyde 0.6g. (2.57 mmol,1 eq), anhydrous THF (15 mL), anhydrous ACN (15 mL), 0.520 g.(2.8 mmol, 1.1 eqs) anhydrous MgBr2, and RM stirred at RT for 30 min. To the RM was added 2.5 mL(14 mmol,5.4 eqs) of anhydrous triethylamine, 10 mL of anhydrous THF, RM cooled at (-20^0 C), and 0.95 g.(2.5 mmol,1 eq) of bromopenam was added. RM stirred at (-20^0 C) for 6 hours. At the same temperature, 3 mL (3 mmol,1.15 eqs) of acetic anhydride was added, RM stirred for 15 min and kept at 0^0 C for 12 hours, evaporated to dryness, residue extracted with 5x 80 mL ethyl acetate. Organic solvent evaporated, and residue purified an a silicagel column (solvent hexane:ethyl acetate 4:1), gave 0.880 g.(52%) of the title compound, yellowish crystals, m.p.141-3^0 C, $(M+H)^+$=661.6.

Step 7: (5R,6Z)-7-oxo-6-(4H-thieno[2',3':4,5]thiopyrano[2,3-b]pyridin-2-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (Sodium salt)

A solution of 4-nitrobenzyl(5R)-6-[(acetyloxy)(4H-thieno[2',3':4,5]thiopyrano[2,3b]pyridin-2-yl) methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0.]hept-ene-2carboxylate 0.8g.(1.21 mmol, 1 eq) in 40 mL THF and 40 mL phosphate buffer solution (pH=6.36) was hydrogenated at 40 psi for 3 hours in the presence of 0.4g. Palladium on Carbon 10% catalyst. RM filtrated trough celite pad, filtrate adjusted to pH=8.0, concentrated in vacuo, residue purified on a reverse-phase column (amberlite), using 5%..10% ACN/water mixture as solvent, gave 0.103g.(21%) of the title compound, reddish crystals, m.p.362.4^0 C, $(M+H)^+$= 409.5.

1H NMR: (DMSO-d_6) δ 4.12(s,2H), 6.49 (s,1H), 6.53(s,1H);7.22(d,1H);7.34 (s,1H);7.41 9s,1H), 7.76 (t,1H);8.28 (d,1H).

Example 65

Preparation of (5*R*,6*Z*)-6-[(5-methyl-7,8-dihydro-6*H*-cyclopenta[e][1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-

carboxylic acid, sodium salt

Step 1: Preparation of (8-Methyl-6,7-dihydro-5H-cyclopenta[d][1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-methanol

To a round bottomed flask was loaded 3.78 grams of 2-acetylcyclopentanone, 3.52 grams of (5-Amino-1H-[1,2,4]triazol-3-yl)-methanol and 50ml 2-methoxyethanol. The mixture was refluxed for 18 hours. Then it was cooled down to 23°C and concentrated to 5ml. Then 50ml ethyl ether was added and the precipitate was filtered and vacuum dried to yielded 2.0 grams of product. This compound was used directly for the next step. MS: 205.2(M+H). H-NMR(DMSO): δ 5.55(t, 1H, OH, J= 6.2Hz), 4.63(d, 2H, J= 6.2Hz), 3.28 (m, 2H), 3.02 (t, 2H, CH2, J= 6.8Hz), 2.51 (s, 3H, CH3), 2.27 (m, 2H, CH2).

Step 2: Preparation of 8-Methyl-6,7-dihydro-5H-cyclopenta[d][1,2,4]triazolo[1,5-a]pyrimidine-2-carbaldehyde

To a round bottomed flask was loaded 0.17ml of DMSO and 1 ml dichloromethane. The mixture was cooled to –50~-60°C. Then a mixture of 0.1ml oxallyl chloride and 2ml dichloromethane was injected in into the flask all at once. The mixture was stirred at the same temperature for another 5 minutes. Then 0.174 grams of (8-Methyl-6,7-dihydro-5H-cyclopenta[d][1,2,4] triazolo [1,5-a]pyrimidin-2-yl)-methanol in 2 ml dichloromethane was added within 2 minutes. The mixture was stirred at –50~-60°C for fifteen minutes and 0.7 ml triethylamine was next added. After another five minutes the reaction media was warmed up to 23°C and a mixture of 20ml water and 200ml dichloromethane was added. The organic layer was dried over magnesium sulfate. Filter off the drying agent and concentrate the filtrate yielded 0.153 grams of product (89%). MS: 203.1(M+H). H-NMR(CDCl3): δ 10.24(s, 1H), 3.49(m, 2H), 3.15(m, 2H), 2.67 (s, 3H, CH3), 2.44 (m, 2H, CH2).

Step 3: Preparation of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(5-methyl-7,8-dihydro-6*H*-cyclopenta[*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

8-Methyl-6,7-dihydro-5H-cyclopenta[d][1,2,4]triazolo[1,5-a]pyrimidine-2-carbaldehyde (153 mg, 0.75 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-

thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (385 mg, 1 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 200 mg, 42%; (M+H) 631.

Step 4: Preparation of (5*R*,6*Z*)-6-[(5-methyl-7,8-dihydro-6*H*-cyclopenta[*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5*R*)-6-[(acetyloxy)(5-methyl-7,8-dihydro-6*H*-cyclopenta[*e*] [1,2,4]triazolo [1,5-*a*]pyrimidin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (200 mg, 0.31 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and phophate buffer (6.5 pH) (20 ml) and hydrogenated over Pd/C (10%) (200 mg) under 40 psi pressure. At the end, reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 15 mg, 13%; as yellow crystals; mp 250^{0}C (Dec); (M+H+Na) 378 .^{1}H NMR (DMSO-d_6) δ 6.80 (s, 1H), 6.76(s, 1H), 6.25(s, 1H), 3.24(m, 2H), 2.96 (m, 2H), 2.49(s, 3H), 2.25(m, 2H).

Example 66

Preparation of (5*R*,6*Z*)-6-{[7-(ethoxycarbonyl)-6,7,8,9-tetrahydropyrido[3,4-e][1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl]methylene}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 2-Hydroxymethyl-8,9-dihydro-6H-1,3,4,7,9b-pentaaza-cyclopenta[a]naphthalene-7-carboxylic acid ethyl ester

To a round bottomed flask was loaded 8.56 grams of 4-oxo-piperidine-1-carboxylic acid ethyl ester, 10.3 ml of dimethylformamide dimethylacetal, and the mixture was refluxed at 90°C for two hours. Then it was poured into 75 ml water and extracted with 2x250ml dichloromethane. The combined organic layers was washed with 50ml brine and dried over magnesium sulfate. Filter off the drying agent and concentrate gave 28 grams of 3-Dimethylaminomethylene-4-oxo-piperidine-1-carboxylic acid ethyl ester. This material (12.8 grams) was then loaded into a round bottomed flask along with 3.42 grams of (5-Amino-1H-[1,2,4]triazol-3-yl)-methanol and 100ml 2-methoxyethanol. The mixture was refluxed for 18 hours. Then it was cooled down to 23°C and concentrated to 5ml. Then 50ml ethyl ether was added and the precipitate was filtered and vaccum dried to yielded 1.5 grams of product. MS: 278.1(M+H). H-NMR(CDCL3): δ 8.60(s, 1H), 4.98(s, 2H), 4.78(s, 2H, CH2), 4.22(q, 2H, J= 4.8Hz), 3.75 (t, 2H, CH2, J= 4Hz), 3.51 (t, 2H, J= 4Hz), 1.32 (m, 3H, CH3, J= 4.8Hz).

Step 2: Preparation of 2-Formyl-8,9-dihydro-6H-1,3,4,7,9b-pentaaza-yclopenta[a]naphthalene-7-carboxylic acid ethyl ester

2-Hydroxymethyl-8,9-dihydro-6H-1,3,4,7,9b-pentaaza-cyclopenta[a]naphthalene-7-carboxylic acid ethyl ester (831 mg, 3 mmol) was converted to 2-formyl-8,9-dihydro-6H-1,3,4,7,9b-pentaaza-yclopenta[a]naphthalene-7-carboxylic acid ethyl ester (690 mg, 89% Yield) by the procedure outlined in example 22, (step 2).

MS: 276.1(M+H). H-NMR(CDCl3): δ 10.24(s, 1H), 8.76(s, 1H), 4.86(s, 2H), 4.23 (q, 2H, CH2, J= 7.2Hz), 4.13 (t, 2H, CH2, J= 7.2Hz) 3.39 (t, 2H, CH2, J= 5.7Hz), 1.34 (t, 3H, CH3, J= 7.2Hz).

Step 3: ethyl 2-[(ac tyloxy)((5*R*)-6-bromo-2-{[(4-nitrobenzyl)oxy]carbonyl}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-8,9-dihydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine-7(6*H*)-carboxylate

2-formyl-8,9-dihydro-6H-1,3,4,7,9b-pentaaza-yclopenta[a]naphthalene-7-carboxylic acid ethyl ester (550 mg, 2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 220 mg, 15%; (M+H) 703.

Step 4: Preparation of (5*R*,6*Z*)-6-{[7-(ethoxycarbonyl)-6,7,8,9-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl]methylene}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Ethyl 2-[(acetyloxy)((5*R*)-6-bromo-2-{[(4-nitrobenzyl)oxy]carbonyl}-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-8,9-dihydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine-7(6*H*)-carboxylate (220 mg, 0.28 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and phophate buffer (6.5 pH) (20 ml) and hydrogenated over Pd/C (10%) (200 mg) under 40 psi pressure. At the end, reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions

containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 15 mg, 2%; Yellow crystals; mp >250^{0}C (Dec); (M+H+Na) 449 .^{1}H NMR (DMSO-d_6) □ 8.61 (s, 1H), 7.01(s, 1H), 6.90(s, 1H), 6.44(s, 1H), 4.74(m, 2H, CH2), 4.13 (q, 2H, J= 5.4Hz), 3.84(s, m, 2H, CH2), 1.22(t, 3H, CH3, J= 5.7Hz).

Example 67

Preparation of (5*R*,6*Z*)-6-(8',9'-dihydro-6'*H*-spiro[1,3-dioxolane-2,7'-[1,2,4]triazolo[1,5-*a*]quinazolin]-2'-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 2-Hydroxymethyl-8,9-dihydro-6H-[1,2,4]triazolo[1,5-a]quinazolin-7-ethylene ketal

To a round bottomed flask was loaded 15.6 g of 1,4-cyclohexadione *mono*-ethylene ketal, 11.9 g of dimethylformamide dimethylacetal, and the mixture was refluxed at 90°C for two hours. Then it was poured into 75 ml water and extracted with 2x250ml dichloromethane. The combined organic layers was washed with 50ml brine and dried over magnesium sulfate. Filter off the drying agent and concentrate gave 28 grams of 3-Dimethylaminomethylene-4-oxo-cyclohexane. The crude product was then loaded into a round bottomed flask along with 11.9 grams of (5-Amino-1H-[1,2,4]triazol-3-yl)-methanol and 100ml 2-methoxyethanol. The mixture was refluxed for 18 hours. Then it was cooled down to 23°C and concentrated to 5ml. Then 50ml ethyl ether was added and the precipitate was filtered and vaccum dried to yielded 2.0 grams (8% Yield) of product. MS: 263 (M+H). H-NMR(CDCL3): δ 8.51(s, 1H), 5.17(s, 2H, CH2), 4.08(s, 4H, OCH2CH2O), 3.42(t, 2H, CH2, J= 5.1Hz), 3.07 (s, 2H, CH2) , 2.15 (t, 3H, CH3, J= 5.1Hz).

Step 2: Preparation of 7-ethyleneketal-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazoline-2-carbaldehyde

To a round bottomed flask was loaded 5ml of DMSO and 5 ml dichloromethane. The mixture was cooled to –50~-60°C. Then a mixture of 1 ml oxallyl chloride and 5ml dichloromethane was injected in into the flask all at once. The mixture was stirred at the

same temperature for another 5 minutes. 2-Hydroxymethyl-8,9-dihydro-6H-[1,2,4]triazolo[1,5-a]quinazolin-7-ethylene ketal (1.31 g, 5 mmol) in 20 ml dichloromethane was added within 2 minutes. The mixture was stirred at –50~-60°C for fifteen minutes and 0.7 ml triethylamine was next added. After another five minutes the reaction media was warmed up to 23°C and a mixture of 20ml water and 200ml dichloromethane was added. The organic layer was dried over magnesium sulfate. Filter off the drying agent and concentrate the filtrate yielded 910 mg of product (70%). MS: 261(M+H). H-NMR(CDCl3): δ 10.26(s, 1H), 8.66(s, 1H), 4.08(s, 4H, OCH2CH2O), 3.49(t, 2H, J= 6.9Hz), 3.11(s, 2H), 2.18 (t, 3H, CH3, J= 6.9Hz), 2.44 (m, 2H, CH2).

Step 3: Preparation of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(8',9'-dihydro-6'*H*-spiro[1,3-dioxolane-2,7'-[1,2,4]triazolo[1,5-*a*]quinazolin]-2'-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

7-Ethyleneketal-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazoline-2-carbaldehyde (780 mg, 3 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.15g g, 3 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 300 mg, 15%; (M+H) 688.8.

Step 4: Preparation of Preparation of (5*R*,6*Z*)-6-(8',9'-dihydro-6'*H*-spiro[1,3-dioxolane-2,7'-[1,2,4]triazolo[1,5-*a*]quinazolin]-2'-ylmethylene)-7-oxo-4-thia-1-

azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5*R*)-6-[(acetyloxy)(8',9'-dihydro-6'*H*-spiro[1,3-dioxolane-2,7'-[1,2,4]triazolo[1,5-*a*]quinazolin]-2'-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (300 mg, 0.43 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and phophate buffer (6.5 pH) (20 ml) and hydrogenated over Pd/C (10%) (200 mg) under 40 psi pressure. At the end, reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 15 mg, 9%; Yellow crystals; mp >250^{0}C (Dec); (M+H+Na) 435.9 .^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 6.97(s, 1H), 6.85(s, 1H), 6.38(s, 1H), 4.05 (s, 4H, OCH2CH2O), 3.28(m, 2H), 3.07 (s, 2H), 2.13(t, 3H, CH3, J= 4.8Hz).

Example 68

Preparation of (5*R*,6*Z*)-6-[(5-methyl-6,7,8,9-tetrahydro[1,2,4]triazolo[1,5-*a*]quinazolin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of (5-Methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazolin-2-yl)-methanol:

To a round bottomed flask was loaded 4.2 grams of 2-acetylcyclohexanone, 3.52 grams of (5-Amino-1H-[1,2,4]triazol-3-yl)-methanol and 50ml 2-methoxyethanol. The mixture was refluxed for 18 hours. Then it was cooled down to 23°C and concentrated to 5ml. Then 50ml ethyl ether was added and the precipitate was filtered and vacuum dried to yielded 3.32 grams of product Yield. 49%. This compound was used directly for the next step. MS: 219.2(M+H). H-NMR(DMSO): δ 5.49(t, 1H, OH, J= 6Hz), 4.61(d, 2H, J= 6Hz), 3.24 (m, 2H), 2.93 (m, 2H), 2.69 (s, 3H), 2.52 (s, 2H), 1.84 (m, 4H).

Step 2: Preparation of 5-Methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazoline-2-carbaldehyde

To a round bottomed flask was loaded 1ml of DMSO and 5 ml dichloromethane. The mixture was cooled to –50~-60°C. Then a mixture of 1 ml oxallyl chloride and 2ml dichloromethane was injected in into the flask all at once. The mixture was stirred at the same temperature for another 5 minutes. Then 0.218 grams of (5-Methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazolin-2-yl)-methanol in 2 ml dichloromethane was added within 2 minutes. The mixture was stirred at –50~-60°C for fifteen minutes and 0.7 ml triethylamine was next added. After another five minutes the reaction media was warmed up to 23°C and a mixture of 20ml water and 200ml dichloromethane was added. The organic layer was dried over magnesium sulfate. Filter off the drying agent and concentrate the filtrate yielded 0.216 grams of product (99%). MS: 217.1(M+H). H-NMR(CDCl3): δ 10.20(s, 1H), 3.23(m, 2H), 2.78 (m, 2H) 2.63 (s, 3H, CH3), 2.00 (m, 4H),

Step 3: Preparation of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(5-methyl-6,7,8,9-tetrahydro[1,2,4]triazolo[1,5-*a*]quinazolin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-

azabicyclo[3.2.0]hept-2-ene-2-carboxylate

5-Methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[1,5-a]quinazoline-2-carbaldehyde (432 mg, 2 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (1.2 g, 3.0 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereomers were taken to the next step. Pale yellow amorphous solid; Yield: 600 mg, 47%; (M+H) 644.7.

<u>Step 4:</u> Preparation of Preparation of (5*R*,6*Z*)-6-[(5-methyl-6,7,8,9-tetrahydro[1,2,4]triazolo[1,5-*a*]quinazolin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5*R*)-6-[(acetyloxy)(5-methyl-6,7,8,9-tetrahydro[1,2,4]triazolo[1,5-*a*]quinazolin-2-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (600 mg, 0.93 mmol) was dissolved in THF (20 mL) and acetonitrile (20 mL) and phophate buffer (6.5 pH) (20 ml) and hydrogenated over Pd/C (10%) (200 mg) under 40 psi pressure. At the end, reaction mixture was filtered, cooled to 3 °C, and 0.1 N NaOH was added to adjust the pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give a yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 L) and latter with 10% acetonitrile:water. The fractions containing the product were collected and concentrated under reduced pressure at room temperature. The yellow solid was washed with acetone, filtered and dried. Yield: 37 mg, 11%; as yellow

crystals; mp 250°C (Dec); (M+H+Na) 392 .^{1}H NMR (DMSO-d_6) δ 6.90 (s, 1H), 6.85(s, 1H), 6.28(s, 1H), 2.98(m, 2H), 2.77 (m, 2H), 2.55(m, 3H), 1.78(m, 4H).

Example 69

Preparation of (5*R*,6*Z*)-6-[(5-methoxy-7,8-dihydro-6*H*-cyclopenta[e]imidazo[1,2-*a*]pyrimidin-2-yl)methylene]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 4-methoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine (SM:Ross, L. O.; Goodman, L.; Baker, B. R. J. Am. Chem. Soc. 1959, 81, 3108)

5.3 grams of 4-chloro-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine was dissolved in 200ml xylene and 30 ml absolute methanol. Then 5.4 gram for sodium methoxide was added and the mixture was refluxed for 3 hours. Then the solvent was removed in vacuo and 100ml water was added to the residue. Filter and wash the cake with water (50ml). The solid was further vacuumed to dry for several hours. The desired product weighed 4.8 gram (98% yield). Mp: 133.8~134.9° C.; MS: 166.2.0 (M+H)

Step 2: Preparation of 5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester

4.8 gram (29mmol) 4-ethoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine was dissolved in 100 ml dry THF. Bromopyruvate (5.4ml,) was then added dropwise with in five minutes. The mixture was stirred at 23oC for one hour. It was then filtered and washed with ether to give 8.7 gram of solid. This solid was then dissolved in 50ml ethanol and refluxed for two hours. The reaction mixture was cooled to room temperature and partitioned between 350ml chloroform and 200 ml saturated sodium bicarbonate. The organic layer was separated and dried over magnesium sulfate. Filter off the drying agent and concentrate to give 5.3 gram of product (70% Yield).
MP: 105-106°C. (M+H) 262.

Step 3: Preparation of 5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-

carbaldehyd

5.2 grams (19.8 mmol) 5-methoxy-7, 8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester was dissolved in 40 ml dichloromethane and then cooled to –78oC. DIBAL (1 M, 30 ml, 1.5 eq.) was then added within five minutes. The reaction media was then quenched with 2ml ethanol and partitioned between 350ml dichloromethane and 100 ml 1 N sodium hydroxide. The aqueous layer was washed with another 150ml chloroform and the combined organic layer was dried over magnesium sulfate and filtered and concentrated to give the corresponding alcohol. The alcohol is then dissolved in 150ml dichloromethane and 10 grams of manganese dioxide is then added. The mixture was stirred at 23 oC for two hours. The reaction mixture was then filtered through a pad of celite and concentrated to give 1.1 gram (68%) of the desired aldehyde. MP: 235.2~236.3° C; MS: 218.1(M+H)

Step 4: Preparation of 6-[acetoxy-(5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

A 30 ml acetonitrile solution of 5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carbaldehyde (660 mg, 3mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.l55 gram, 1 eq.) was injected within a minute and the reaction mixture was then cooled to –20° C. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml, eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 1.8gram product. (93% Yield); MP: 118.7~119.1 °C; MS: 645.9(M+H)

Step 5: Preparation of 6-(5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6-[acetoxy-(5-methoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (966 mg, 1.4 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 100 mg of product was collected.

MP: >250^0 C

H-NMR: (300 MHz, D_2O) δ 10.12 (s, 1H), 9.29(s, 1H), 8.81(s, 1H), 8.78(s, 1H), 6.19 (s, 3H), 5.36(m, 2H), 5.05 (m, 2H), 4.43 (m, 2H).; MS: 371.2 (M+H).

Example 70

Preparation of (5*R*,6*Z*)-6-({5-[2-(benzyloxy)ethoxy]-7,8-dihydro-6*H*-cyclopenta[e]imidazo[1,2-*a*]pyrimidin-2-yl}methylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of 4-benzyloxyethoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine

(SM:Ross, L. O.; Goodman, L.; Baker, B. R. J. Am. Chem. Soc. 1959, 81, 3108)

To stirred suspension of NaH (60% 552 mg) in THF 2-benzyloxyethanol (3.38 g, 20 mmol) was slowly added at room temperature. After the addition, , 3.28 grams (19.4 mmol) of 4-chloro-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine was dissolved in 200ml THF and added to it and the mixture was refluxed for 3 hours. Then the solvent was removed in vacuo and 100ml water was added to the residue. The product was extracted with chloroform; washed well with water and dried over anhydrous $MgSO_4$. It was filtered and concentrated. Low melting solid; Yield: 4.2 gram (73%); (M+H) 286.1

Step 2: Preparation of 5-benzyloxyethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester

6.0 gram (21mmol) of 4-benzyloxyethoxy-6,7-dihydro-5H-cyclopentapyrimidin-2-ylamine

was dissolved in 100 ml dry THF. Bromopyruvate (8 ml,) was then added dropwise with in five minutes. The mixture was stirred at 23oC for one hour. It was then filtered and washed with ether to give a solid. This solid was then dissolved in 50ml ethanol and refluxed for two hours. The reaction mixture was cooled to room temperature and partitioned between 350ml chloroform and 200 ml saturated sodium bicarbonate. The organic layer was separated and dried over magnesium sulfate. Filter off the drying agent and concentrate to give 5.36 gram of product (67% Yield).
(M+H) 382.1

Step 3: Preparation of 5-benzyloxyethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carbaldehyde

3.81 grams (10 mmol) 5-benzyloxyethoxy-7, 8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carboxylic acid ethyl ester was dissolved in 40 ml dichloromethane and then cooled to –78oC. DIBAL (1 M, 30 ml, 1.5 eq.) was then added within five minutes. The reaction media was then quenched with 2ml ethanol and partitioned between 350ml dichloromethane and 100 ml 1 N sodium hydroxide. The aqueous layer was washed with another 150ml chloroform and the combined organic layer was dried over magnesium sulfate and filtered and concentrated to give the corresponding alcohol. The alcohol is then dissolved in 150ml dichloromethane and 10 grams of manganese dioxide is then added. The mixture was stirred at 23 oC for two hours. The reaction mixture was then filtered through a pad of celite and concentrated to give 2.25 gram (67%) of the desired aldehyde. MS: 338(M+H)

Step 4: Preparation of 6-[acetoxy-(5-[2-(benzyloxy)emethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

A 30 ml acetonitrile solution of 5-benzyloxyethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacene-2-carbaldehyde (676 mg, 2mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) was injected within a minute and the reaction mixture was then cooled to –20oC. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml,

eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 1.05 gram product. (68% Yield); MS: 765.8(M+H)

Step 5: Preparation of Preparation of (5*R*,6*Z*)-6-({5-[2-(benzyloxy)ethoxy]-7,8-dihydro-6*H*-cyclopenta[*e*]imidazo[1,2-*a*]pyrimidin-2-yl}methylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6-[acetoxy-(5-[2-(benzyloxy)emethoxy-7,8-dihydro-6H-3,4,8b-triaza-as-indacen-2-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (966 mg, 1.2 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 100 mg of product was collected. MP: >250^0 C; H-NMR(DMSO): □ 7.66(s, 1H), 7.36(s, 1H), 7.08(m, 5H), 6.87(s, 1H), 6.85(s, 1H), 4.37 (m, 2H), 4.29 (m, 2H, CH2), 3.65 (m, 2H, CH2), 2.73 (m, 2H, CH2), 2.46 (m, 2H, CH2), 2.02 (m, 2H, CH2).

MS: 491.1 (M+H).

Example 71

Preparation of (5*R*,6*Z*)-6-(2,3-dihydro[1,3]thiazolo[3,2-*a*]benzimidazol-6-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of (2,3-Dihydro-benzo[4,5]imidazo[2,1-b]thiazol-7-yl)-methanol

To a round bottomed flask was added 2.83 grams of 2-Thioxo-2,3-dihydro-1H-benzoimidazole-5-carboxylic acid methyl ester, 2.55 grams of dibromoethane and 50ml DMF and 50ml ethanol. The mixture was refluxed for 10 hours. Then it was

concentrated to dry on a rotary evaporator. The solid was next dissolved in 100ml THF and 20 ml of 1M $LiAlH_4$ (in THF) was next injected within five minutes. The reaction media was stirred at room temperature for one hour. Ethanol was next added (~10ml), followed by 50ml 2N HCl. The aqueous layer was adjusted to basic Ph=14 with 10N sodium hydroxide. The aqueous was extracted with 2x500ml ethyl acetate. The combined organic layers was dried over magnesium sulfate. Filter off the drying agent and cocentrate yielded 2.04 grams (60%) product. MS: 207.0(M+H). H-NMR(DMSO): □ 7.34(m, 2H), 7.08 (m, 1H), 5.15(m, 1H, OH), 4.53 (m, 2H, CH2), 4.34 (m, 2H, CH2), 4.00 (m, 2H, CH2).

Step 2: Preparation of 2,3-Dihydro-benzo[4,5]imidazo[2,1-b]thiazole-7-carbaldehyde

To a pre-cooled (-50~-60oC) mixture of 1.7ml DMSO and 5ml dichloromathane was injected a 20ml dichloromethane solution of 1ml oxallyl chloride within five minutes. The mixture was stirred for another five minutes at the same temperature. Then 1.9 grams of 2,3-Dihydro-benzo[4,5]imidazo[2,1-b]thiazol-7-yl)-methanol in a mixture of 20ml dichloromethane and 20 ml THF was injected within 2 minutes. The mixture was kept stirred at –50~-60°C for 15 minutes. Then 7ml triethylamine was injected all at once and after another 5minutes the cooling bath was removed and the reaction was warmed up to room temperature by itself. Water (100ml) was next added and the reaction media was extracted with 2x200ml ethyl acetate. The combined organic layers was dried over magnesium sulfate. Filter off the drying agent and concentrate gave 1.2 grams product (64%). MS: 205.0(M+H). H-NMR(CDCl3): □ 9.98(m, 1H), 7.67 (m, 2H), 7.17 (m, 1H), 4.33(m, 2H), 3.99 (m, 2H, CH2).

Step 3: Preparation of 6-[Acetoxy-(2,3-dihydro-benzo[4,5]imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester

A 30 ml acetonitrile solution of 2,3-Dihydro-benzo[4,5]imidazo[2,1-b]thiazole-7-carbaldehyde (610 mg, 2mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) was injected within a minute and the reaction mixture was then cooled

to –20oC. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml, eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 690 mg product. (54% Yield); MS: 630.8(M+H)

Step 4: Preparation of (5*R*,6*Z*)-6-(2,3-dihydro[1,3]thiazolo[3,2-*a*]benzimidazol-6-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

6-[Acetoxy-(2,3-dihydro-benzo[4,5]imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (690 mg, 1.1 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 32 mg of product (Yield 3%) was collected. MP: >250^0 C; H-NMR(D_2O): δ 7.08(m, 6H), 7.36(s, 1H), 4.05(m, 2H), 3.90(b, 1H); MS: 358.3 (M+H).

Example 72

Preparation of (5*R*,6*Z*)-6-(3,4-dihydro-2*H*-[1,3]thiazino[3,2-*a*]benzimidazol-7-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of (3,4-Dihydro-2H-1-thia-4a,9-diaza-fluoren-6-yl)-methanol

Step 1: Preparation of (2,3-Dihydro-benzo[4,5]imidazo[2,1-b]thiazol-7-yl)-methanol

To a round bottomed flask was added 4.06 grams of 2-Thioxo-2,3-dihydro-1H-benzoimidazole-5-carboxylic acid methyl ester, 4.04 grams of 1,3-dibromopropane and 50ml DMF and 50ml ethanol. The mixture was refluxed for 10 hours. Then it was concentrated to dry on a rotary evaporator. The solid was next dissolved in 100ml THF and 20 ml of 1M $LiAlH_4$ (in THF) was next injected within five minutes. The reaction

media was stirred at room temperature for one hour. Ethanol was next added (~10ml), followed by 50ml 2N HCl. The aqueous layer was adjusted to basic Ph=14 with 10N sodium hydroxide. The aqueous was extracted with 2x500ml ethyl acetate. The combined organic layers was dried over magnesium sulfate. Filter off the drying agent and cocentrate yielded 3 grams (68%) product. NMR(DMSO): δ 7.91(m, 3H), 4.13 (m, 2H), 3.93(s, 1H), 3.23 (m, 2H, CH2), 2.48 (m, 2H, CH2). MS: 221.0(M+H).

Step 2: Preparation of 3,4-Dihydro-2H-1-thia-4a,9-diaza-fluorene-6-carbaldehyde

To a round bottomed flask was loaded 1.1 grams of (3,4-Dihydro-2H-1-thia-4a,9-diaza-fluoren-6-yl)-methanol , 6 grams of manganese dioxide and 250 ml chloroform. The mixture was stirred for one hour at room temperature and then filtered through a pad of celite. This yielded 0.67 grams of product (61%). MS: 219.0(M+H). H-NMR(CDCl3): δ 10.04(s, 1H), 7.67 (m, 3H), 4.25 (m, 2H), 3.27(m, 2H), 2.50 (m, 2H).

Step 3: Preparation of 4-nitrobenzyl (5*R*)-6-[(acetyloxy)(3,4-dihydro-2*H*-[1,3]thiazino[3,2-*a*]benzimidazol-7-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

A 30 ml acetonitrile solution of 3,4-Dihydro-2H-1-thia-4a,9-diaza-fluorene-6-carbaldehyde (660 mg, 3mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.15 g, 3 mmol) was injected within a minute and the reaction mixture was then cooled to –20oC. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml, eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 690 mg product. (36% Yield); MS: 644.9(M+H)

Step 4: Preparation of (5*R*,6*Z*)-6-(3,4-dihydro-2*H*-[1,3]thiazino[3,2-*a*]benzimidazol-7-ylmethylene)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5*R*)-6-[(acetyloxy)(3,4-dihydro-2*H*-[1,3]thiazino[3,2-*a*]benzimidazol-7-

yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (700 mg, 1.1 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 75 mg of product (Yield 18%) was collected. MP: >250^0 C; H-NMR(D_2O): δ 7.08(m, 6H), 3.70(m, 2H), 4.05(m, 2H), 3.13(m, 2H), 2.22(m, 2H); MS: 372.1(M+H).

Example 73

Preparation of (5*R*,6*Z*)-7-oxo-6-([1,3]thiazolo[3,2-*a*]benzimidazol-6-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of Benzo[4,5]imidazo[2,1-b]thiazole-6-carboxylic acid methyl ester

To a round bottomed flask was loaded with 3.3 grams of 2-Thioxo-2,3-dihydro-1H-benzoimidazole-5-carboxylic acid methyl ester, 4.5ml alpha-bromodiethylacetal, 50ml DMF. The mixture was refluxed for 10 hours. Then is was poured into 10% sat. sodium bicarbonate (100ml) and extracted with 2x100ml ethyl acetate. The combined organic layers were dried over magnesium sulfate. Filter off the drying agent, concentrate to dry, flash column chromatography using 10~30% ethyl acetate/hexane yielded 1.16 grams (32%) crude product. MS: 233.1(M+H). H-NMR(DMSO): δ 7.78(m, 5H), 2.04 (s, 3H, CH3).

Step 2: Preparation of Benzo[4,5]imidazo[2,1-b]thiazole-6-carbaldehyde

To a round bottomed flask was loaded 1.16 grams of (3,4-Dihydro-2H-1-thia-4a,9-diaza-fluoren-6-yl)-methanol , 25 grams of manganese dioxide and 250 ml chloroform. The mixture was stirred for one hour at room temperature and then filtered through a pad of celite. This yielded 0.42 grams of product (42%). MS: 203.0(M+H). H-NMR(CDCl3): δ 10.10(ss, 1H), 8.24 (ss, 1H), 7.85 (m, 3H), 6.96 (m, 1H).

Step 3: Preparation of 4-nitrob nzyl (5*R*)-6-[(acetyloxy)([1,3]thiazolo[3,2-*a*]benzimidazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

A 30 ml acetonitrile solution of benzo[4,5]imidazo[2,1-b]thiazole-6-carbaldehyde (404 mg, 2mmol) was added 1.03 gram of magnesium bromide etherate. The mixture was stirred at 23oC for half an hour. Then a 30ml dry THF solution of the 6-Bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (770 mg, 2 mmol) was injected within a minute and the reaction mixture was then cooled to –20oC. Triethylamine (0.7 ml, eq.) was then injected and the reaction mixture was stirred for five hours at –20oC. Then acetic anhydride (0.377 ml, eq.) was injected and the reaction mixture was left at zero degree for 18 hours. The reaction media was then diluted with 400ml ethyl acetate and washed with 100 ml 5% citric acid, 100 ml saturated sodium bicarbonate, and 100ml brine. The organic layer was then dried over magnesium sulfate, filtered and concentrated. Flash column chromatography using 20% ethyl acetate in hexane gave 630 mg product. (50% Yield); MS: 631.9(M+H)

Step 4: Preparation of (5*R*,6*Z*)-7-oxo-6-([1,3]thiazolo[3,2-*a*]benzimidazol-6-ylmethylene)-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

4-nitrobenzyl (5*R*)-6-[(acetyloxy)([1,3]thiazolo[3,2-*a*]benzimidazol-6-yl)methyl]-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (630 mg, 1 mmol) was suspended in 20 ml THF and 20 ml pH=6.5 aqueous phosphate buffer. The mixture was then subjected to 45psi hydrogen for two hours. Then it was filtered through a pad of celite and concentrated in vacuo to remove most of the THF. The solution was then cooled to zero degree and basified to pH=8 with 1 N sodium hydroxide. Then it was purified via reverse phase HPLC using 1 liter of water followed by 5% ~25% acetonitrile and water. Water was then removed through concentrate in vacuo and 33 mg of product (Yield 8%) was collected. MP: >250^0 C; H-NMR(D_2O): δ 6.89(m, 8H), 5.22(s, 2H), 5.02(s, 2H), 4.81(s, 2H).

MS: 378.1(M+H+Na).

Example 74

Preparation of (5*R*,6*Z*)-6-(7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethylene)7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl-5-[(4-oxotetrahydro-2H-pyran-3-yl)oxy]-1H-pyrazole-3-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (7.0 g, 45 mmol) and 24.9 g g of potassium carbonate in 500 ml of acetonitrile was added 8.0 g of 3-bromo-tetrahydro-pyran-4-one, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. 9.0 g (78%) of the desired product was obtainedas a white solid. M.Pt. 121-123^{0}C; (M+H) 255.

Step 2: Preparation of ethyl 7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3] oxazole-2-carboxylate:

A mixture of ethyl-5-[(4-oxotetrahydro-2H-pyran-3-yl)oxy]-1H-pyrazole-3-carboxylate (254 mg, 1 mmol) and methane sulfonic acid (192 mg) in 7 ml of acetic acid and toluene (50 ml) was refluxed for 18 hours using a Dean–Stark trap to remove water. The reaction mixture was allowed to cool to room temperature. The reaction mixture was filtered. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate aqueous bicarbonate solution. The organic layer was washed with water and dried over $MgSO_4$. After removal of the ethyl acetate, the residue was purified by silica gel chromatography eluting with ethyl acetate/hexane to give 120 mg (51%) of the desired product as white solid. Mp; 116-118^0 C; Electrospray-MS m/z 237.0 $(M+H)^+$

Step 3: Preparation of 7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethanol:

To the stirred solution of 7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazole-2-

carboxylate (1.5 g, 6.3 mmol) of in 100 ml of THF was added 1.05 g of lithium borohydride and 1.54 g of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 0.74 g of the desired product (60%). (M+H) 196.

Step 4: Preparation of 7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-carbaldehyde:

To the stirred solution of 7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethanol (1.0 g, 5.1 mmol) in 60 ml of $CHCl_3$ was added 8 g of MnO_2. Th suspension was refluxed for 1.5 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow oil. The product was purified by chromatography. 0.79 g of the product was obtained (80%); (M+H) 193

Step 5: 4-Nitrobenzy (5R)-6-[(acetyloxy)(7,8-dihydro-5H-pyrano[4,3]pyrazolo[5,1-b][1,3]oxazol-2-yl)methyl] -6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-carbaldehyde (600 mg, 3.1 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54 g, 4.6 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g , 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was

washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 1.35 g, 70%; (M+H) 619.

Step 6: Preparation of (5*R*,6*Z*)-6-(7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethylene)7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt & (5*R*,6*E*)-6-(7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethylene)7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4-Nitrobenzy (5R)-6-[(acetyloxy)(7,8-dihydro-5H-pyrano[4,3]pyrazolo[5,1-b][1,3]oxazol-2-yl)methyl] -6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (1.2 g, 1.9 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. In this reaction both E and Z isomers were formed and they were separated by prep. HPLC.

(5*R*,6*Z*)-6-(7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethylene)7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt : Yield 87 mg, (25%); Yellow solid; (M+H+Na) 368.2.

H-NMR (D_2O): 7.04 (1H, s), 7.01 (1H, s), 6.45 (1H, s), 6.09 (1H, s), 4.76 (2H, m), 4.12 (2H, m), 2.96 (2H, m).

(5*R*,6*E*)-6-(7,8-dihydro-5H-pyrano[4,3-d]pyrazolo[5,1-b][1,3]oxazol-2-ylmethylene)7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt : Yield 75 mg, (21%); Yellow solid; (M+H+Na) 368.2.

H-NMR (D_2O): 7.08 (1H, s), 6.81 (1H, s), 6.71 (1H, s), 6.40 (1H, s), 4.68 (2H, m), 4.03 (2H, m), 2.87 (2H, m).

Example 75

Preparation of (5*R*,6*Z*)-7-oxo-6-(5,6,7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazol-2-ylmethylene)-4-thia- -1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt,

Step 1: Preparation of ethyl-5-[(2-oxocyclohexyl)oxy]-1H-pyrazole-3-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (6.25 g, 40 mmol) and 22.1 g of potassium carbonate in 500 ml of acetonitrile was added 6.35 g of 2-chlorocyclohexanone, and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The product was purified by silics-gel column chromatography by eluting it with 1:1 ethyl acetaet;hexane. 4.92 g (49%) of the desired product was obtained as a white solid. M.Pt. 122-124^{0}C; (M+H) 253.

Step 2: Preparation of ethyl 5,6,7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazole-2-carboxylate:

A mixture of ethyl-5-[(2-oxocyclohexyl)oxy]-1H-pyrazole-3-carboxylate (127.6 mg, 0.5 mmol) and methane sulfonic acid (95 mg) in 5 ml of acetic acid and toluene (50 ml) was refluxed for 18 hours using a Dean–Stark trap to remove water. The reaction mixture was allowed to cool to room temperature. The reaction mixture was filtered. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and aqueous bicarbonate solution. The organic layer was washed with water and dried over $MgSO_4$. After removal of the ethyl acetate, the residue was purified by silica gel chromatography eluting with 1:1 ethyl acetate/hexane to give 69.7 mg (59%) of the desired product as white solid. Mp; 55-57^0 C; Electrospray-MS m/z 235.0 $(M+H)^+$

Step 3: Preparation of 5,6, 7,8-tetraihydropyrazolo[5,1-b][1,3]benzoxazol-2-ylmethanol:

To the stirred solution of ethyl 5,6,7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazole-2-carboxylate (3.84 g, 16.4 mmol) of in 100 ml of THF was added 3.05 g of lithium borohydride and 3 ml of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 2.62 g of the desired product (83%). Mpt. 82-84^{0}C; (M+H) 193.

Step 4: Preparation of 5,6, 7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazole-2-carbaldehyde:

To the stirred solution of 5,6, 7,8-tetraihydropyrazolo[5,1-b][1,3]benzoxazol-2-ylmethanol (2.30 g, 11.97 mmol) in 60 ml of $CHCl_3$ was added 10 g of MnO_2. Th suspension was refluxed for 1.5 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow solid. The product was purified by chromatography. 1.95 g of the product was obtained (85.5%); (M+H) 191

Step 5: 4-Nitrobenzy (5R)-6-[(acetyloxy)(5,67,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazol-2-yl)methyl-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

5,6, 7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazole-2-carbaldehyde (589 mg, 3.1 mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54 g, 4.6 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g , 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was

washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 792 mg, 42%; M.pt. 160-162^{0}C; (M+H) 618.

Step 6: Preparation of (5*R*,6*Z*)-7-oxo-6-(5,6,7,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazol-2-ylmethylene)-4-thia- -1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

4-Nitrobenzy (5R)-6-[(acetyloxy)(5,67,8-tetrahydropyrazolo[5,1-b][1,3]benzoxazol-2-yl)methyl-6-bromo-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (318 mg, 0.5 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C (100 mg) at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Yield 150 mg, (76%); Yellow solid; (M+H+Na) 365.2.
H-NMR (D_2O): δ 6.92 (1H, s), 6.91 (1H, s), 6.32 (1H, s), 5.85 (1H, s), 2.59 (4H, m), 1.80 (4H,m).

Example 76

Preparation of (5*R*,6*Z*)-6-{[6-(ethoxycarbonyl)-5,6,7,8-tetrahydropyrazolo[5',1':2,3][1,3]oxazolo[5,4-c]pyridin-2-yl]methylene}-7-oxo-4-thia- -1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Step 1: Preparation of ethyl 3-{[3-ethoxycarbonyl)-1H-pyrazol-5-yl]oxy}-4-oxopiperidine-1-carboxylate:

To the stirred suspension of ethyl 5-hydroxy-1H-pyrazole-3-carboxylate (19.5 g, 127 mmol) and 50.0 g of potassium carbonate in 500 ml of acetonitrile was added 3-bromo–4-oxo-piperidine-1-carboxylic acid ethyl ester (37.45 g, 149 mmol), and refluxed for 16 hours. The reaction mixture was allowed to cool to room temperature, then filtered, the solid was washed with acetonitrile. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and extracted with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The product was purified by silics-gel column chromatography by eluting it with 1:1 ethyl acetaet;hexane. 8.5 g (19%) of the desired product was obtained as an yellow oil. (M+H) 326.

Step 2: Preparation of diethyl 7,8-tetrahydropyrazolo[5' ,1':2,3][1,3]oxazolo[5,4-c]pyridine-2,6(5H)-dicarboxylate:

A mixture of ethyl 3-{[3-ethoxycarbonyl)-1H-pyrazol-5-yl]oxy}-4-oxopiperidine-1-carboxylate (325 mg, 1 mmol) and methane sulfonic acid (95 mg) in 5 ml of acetic acid and toluene (50 ml) was refluxed for 18 hours using a Dean–Stark trap to remove water. The reaction mixture was allowed to cool to room temperature. The reaction mixture was filtered. The filtrate was concentrated to an oil. The residue was dissolved in ethyl acetate and aqueous bicarbonate solution. The organic layer was washed with water and dried over $MgSO_4$. After removal of the ethyl acetate, the residue was purified by silica gel chromatography eluting with 1:1 ethyl acetate/hexane to give 175 mg (57%) of the desired product as an yellow oil Electrospray-MS m/z 308.0 $(M+H)^+$

Step 3: Preparation of ethyl 2-(hydroxymethyl)-7,8-dihydropyrazolo [5' ,1':2,3][1,3]oxazolo[5,4-c]pyridine-6(5H)-carboxylate

To the stirred solution of diethyl 7,8-tetrahydropyrazolo[5' ,1':2,3][1,3]oxazolo[5,4-c]pyridine-2,6(5H)-dicarboxylate (307 mg, 1 mmol) of in 40 ml of THF was added 305 mg of lithium borohydride and 1 ml of methanol. The solution was heated at 40C for 2.5 hour. The reaction was quenched by 1N HCl, and adjusted to pH 1.3 and stirred at room temperature for 1 hour. The reaction mixture was adjusted pH to 8 with k_2CO_3. The reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and concentrated to an oil and column chromatographyed to give 172 mg of the desired product (65%); (M+H) 266.

Step 4: Pr paration of ethyl 2-formyl-7,8-dihydropyrazolo [5' ,1':2,3][1,3]oxazolo [5,4-c]pyridin -6(5H)-carboxylate

To the stirred solution of ethyl 2-(hydroxymethyl)-7,8-dihydropyrazolo [5' ,1':2,3][1,3]oxazolo[5,4-c]pyridine-6(5H)-carboxylate (1.76 g, 6.6 mmol) in 60 ml of $CHCl_3$ was added 10 g of MnO_2. Th suspension was refluxed for 1.5 hour under a nitrogen atmosphere. The reaction mixture was filtered through a pad of Celite. The filtrate was concentrated to give yellow solid. The product was purified by chromatography. 1.43 g of the product was obtained (82%); M.pt: 97-99^{0}C (M+H) 264.

Step 5: Preparation of ethyl 2-[(acetyloxy)(5R)-6-bromo-2-Z{[(4-nitrobenzyl)oxy] carbonyl} -7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-7,8-dihydropyrazolo[5',1':2,3][1,3]oxazolo[5,4-c]pyridine-6(5H)-carboxylate

Ethyl 2-formyl-7,8-dihydropyrazolo [5' ,1':2,3][1,3]oxazolo[5,4-c]pyridine-6(5H)-carboxylate (790 mg, 3. mmol) and the dry THF solution (20 mL) of (5R, 6S)-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid 4-nitro-benzyl ester (1.54 g, 4.6 mmol) were added successively to the dry acetonitrile (15 mL) solution of anhydrous $MgBr_2$: $O(Et)_2$ (2.21 g , 8.5 mmol)under an argon atmosphere at room temperature. After cooling to –20 °C, Et_3N (2.0 mL) was added in one portion. The reaction vessel was covered with foil to exclude light. The reaction mixture was stirred for 2 h at –20 °C and treated with acetic anhydride (1.04 mL) in one portion. The reaction mixture was warmed to 0 °C and stirred for 15 h at 0 °C. The mixture was diluted with ethyl acetate and washed with 5% citric acid aqueous solution, saturated sodium hydrogen carbonate, and brine. The organic layer was dried ($MgSO_4$) and filtered through a pad of Celite. The pad was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography, then the column was eluted with ethyl acetate: hexane (1:1). Collected fractions were concentrated under reduced pressure and the mixture of diastereo isomers were taken to next step. Pale yellow amorphous solid; Yield: 1.67 g, 81%; (M+H) 690.

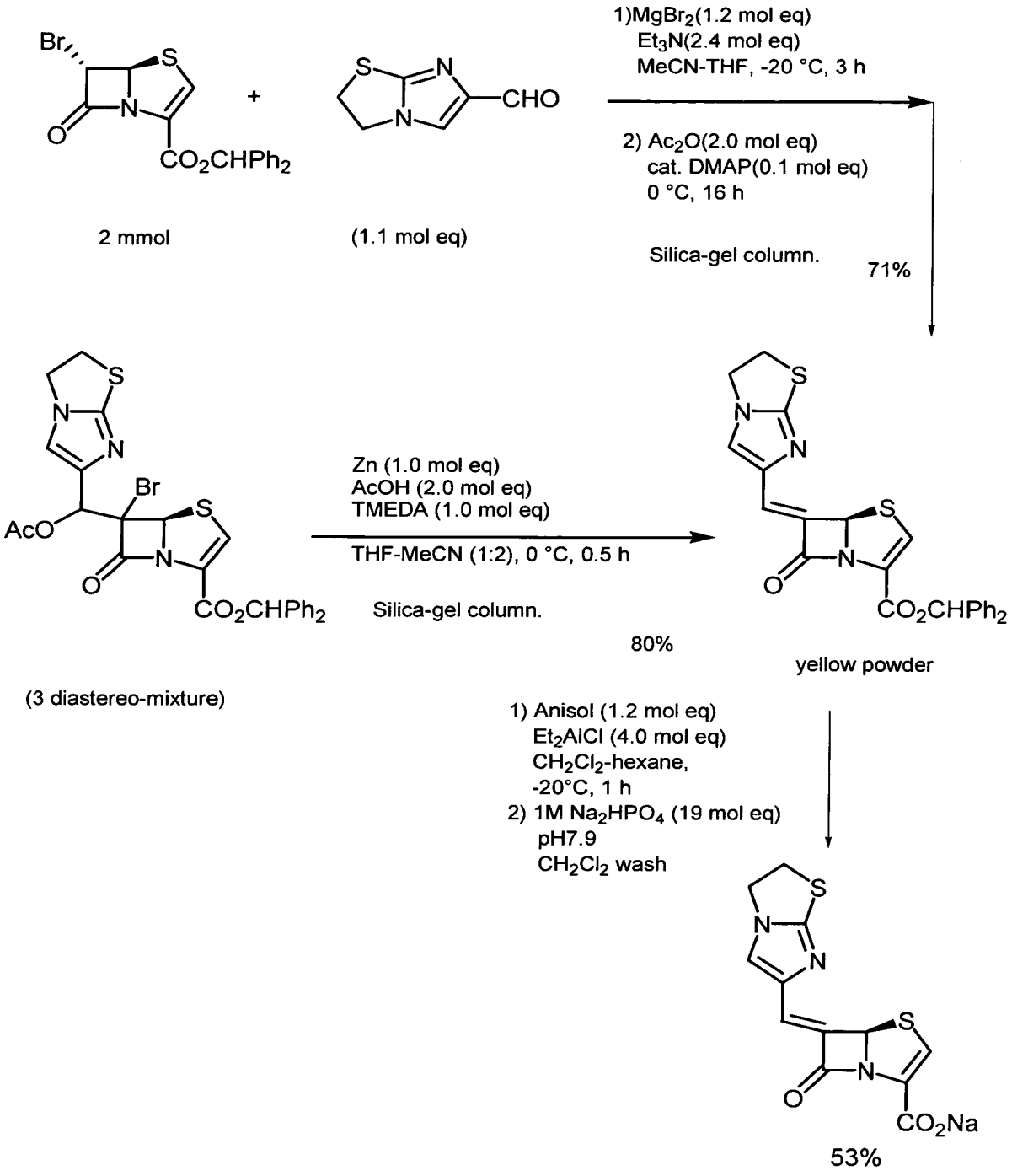
Step 6: Preparation of (5*R*,6*Z*)-6-{[6-(ethoxycarbonyl)-5,6,7,8-tetrahydropyrazolo[5',1':2,3][1,3]oxazolo[5,4-c]pyridin-2-yl]methylene}-7-oxo-4-thia- -1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, sodium salt

Ethyl 2-[(acetyloxy)(5R)-6-bromo-2-Z{[(4-nitrobenzyl)oxy]carbonyl} -7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-en-6-yl)methyl]-7,8-dihydropyrazolo[5',1':2,3][1,3] oxazolo[5,4-c]pyridine-6(5H)-carboxylate (828 mg, 0.5 mmol) was dissolved in THF (20 mL), acetonitrile (10 mL) and 0.5 *M* phosphate buffer (pH 6.5, 28 mL) and hydrogenated over 10% Pd/C (200 mg) at 40 psi pressure. After 4 hrs the reaction mixture was filtered, cooled to 3 °C, and 0.1 *M* NaOH was added to adjust pH to 8.5. The filtrate was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was concentrated under high vacuum at 35 °C to give yellow precipitate. The product was purified by HP21 resin reverse phase column chromatography. Initially the column was eluted with deionized water (2 lits) and latter with 10% acetonitrile: Water. The fractions containing the product were collected and concentrated at reduced pressure at room temperature. The yellow solid was washed with acetone and filtered. Yield 375 mg, (71%); Yellow solid; (M+H+Na) 438.4.

H-NMR (D_2O): δ 6.96 (1H, s), 6.94 (1H, s), 6.41 (1H, s), 6.00 (1H, s), 4.53 (2H, m), 4.13 (2H,q), 3.78 (2H,m), 2.78 (2H, m), 1.21 (3H, t).

Example 77

Preparation of 6-[Acetoxy-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid benzhydryl ester


1)MgBr2(1.2 mol eq)
Et3N(2.4 mol eq)
MeCN-THF, -20 °C, 3 h
2) Ac2O(2.0 mol eq)
cat. DMAP(0.1 mol eq)
0 °C, 16 h
Silica-gel column.
71%
2 mmol
(1.1 mol eq)
Zn (1.0 mol eq)
AcOH (2.0 mol eq)
TMEDA (1.0 mol eq)
THF-MeCN (1:2), 0 °C, 0.5 h
Silica-gel column.
80%
yellow powder
(3 diastereo-mixture)
1) Anisol (1.2 mol eq)
Et2AlCl (4.0 mol eq)
CH2Cl2-hexane,
-20°C, 1 h
2) 1M Na2HPO4 (19 mol eq)
pH7.9
CH2Cl2 wash
53%

5

Step 1: Preparation of 6-[Acetoxy-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid benzhydryl ester

$MgBr_2$ (450.9mg, 1.2 mol eq) was weighed under nitrogen atmosphere, dissolved in dry actonitrile (17.1ml) at room temperature and Benzhydryl 2-[(3S,4R)-3-bromo-4-formylthio-2- oxoazetidin-1-yl]-3-methylbut-2-enoate (832 mg, 2mmol) was added to the mixture. A solution of 2,3-Dihydro-imidazo[2,1-b] thiazole-6-carbaldehyde (339mg, 1.1mol eq) in THF (17.1ml) was added to the mixture at room temperature then stirred and cooled to –20°C. The cold solution was stirred for 3 hours after the addition of Et_3N (0.67ml, 2.4 mol eq) then treated with Ac_2O (0.39ml, 2.0 mol eq) and DMAP (24.4mg, 0.1 mol eq) at the same temperature. Reaction mixture was allowed to warm to 0°C, stirred overnight then diluted with AcOEt (30ml) and 5% aqueous citric acid (20ml). The organic phase was transferred, washed with saturated aqueous sodium bicarbonate and with brine and dried over $MgSO_4$. The resulting residue after evaporation was chromatographed on silica-gel (20g), eluting with 10% AcOEt in $CHCl_3$, to give the product as a yellow amorphous (866mg, 71%).

^{1}H-NMR ($CDCl_3$): δ 1.98 (s, 3H), 3.77-3.82 (m, 2H), 4.09-4.18 (m, 2H), 6.05 (s, 1H x 0.1), 6.23 (s, 1H x 0.4), 6.28 (s, 1H x 0.5), 6.96 (s, 1H), 7.14 (s, 1H), 7.25-7.48 (m, 11H).

Step 2: 6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid benzhydryl ester

A solution of 6-[Acetoxy-(2,3-dihydro-imidazo[2,1-b]thiazol-6-yl)-methyl]-6-bromo-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid benzhydryl ester (827mg, 1.35mmol) in dry THF (5ml) and dry MeCN (10ml) was cooled to 0°C and added consecutively AcOH (155µl, 2.0 mol eq) and Zn (88mg, 1.0 mol eq) and TMEDA (204µl, 1.0 mol eq) under nitrogen atmosphere. Reaction mixture was stirred for 30 min. then evaporated to dryness under reduced pressure. The residue was chromatographed on silica-gel (16g), eluting with 20% AcOEt in $CHCl_3$, to give the product as a yellow powder (511mg, 80%, m.p. 160-161°C).

^{1}H-NMR ($CDCl_3$): δ 3.79 (t, 2H, *J*=7.2Hz), 4.11-4.18 (m, 2H), 6.59 (s, 1H), 6.83 (s, 1H), 6.95 (s, 1H), 7.14 (s, 1H), 7.25-7.48 (m, 10H).

Step 3: (5R), (6Z)-6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)-7-oxo-4- thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid sodium salt

A solution of Anisol (9.5µl, 1.2 mol eq) in dry CH_2Cl_2 (0.5ml) was cooled to -20°C and treated with Et_2AlCl (1mol/L solution in hexane, 0.29ml, 4.0 mol eq) under nitrogen atmosphere followed by titration of the solution of compound 6-(2,3-Dihydro-imidazo[2,1-b]thiazol-6-ylmethylene)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid benzhydryl ester (34.6mg, 0.073mmol) in dry CH_2Cl_2 (2.0ml) over 15min. The reaction mixture was stirred for 1 hour at -10°C, added the 1mol/L aqueous solution of Na_2HPO_4 (507mg, 19 mol eq) and water (1ml) and then stirred for 30 min. at 0°C. Celite (84mg) was added with stirring for additional 30min. and filtered off. The aqueous phase was washed with CH_2Cl_2 (4ml x 2), evaporated to about 5ml under high vacuum and desalted by chromatography on SP207 resin. Eluting with 20% MeCN in water and lyophilizing the active fraction afforded the title compound as a yellow amorphous (12.8mg, 53%).
^{1}H-NMR (D_2O): δ 3.82 (t, 2H, *J*=7.2Hz), 4.18 (t, 2H, *J*=7.2Hz), 6.46 (s, 1H), 6.89 (s, 1H), 6.94 (s, 1H), 7.46 (s, 1H).